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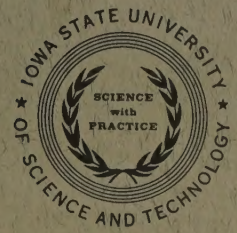

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NOTICE TO SUBSCRIBERS

The name of this journal, the IOWA STATE COLLEGE JOURNAL OF SCIENCE, beginning with this issue (Volume 34, No. 1, August 1959) is changed to

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IOWA STATE JOURNAL OF SCIENCE
Vol. 34 No. 1 August 15, 1959 pp. 1-9

ESTIMATION OF CONSECUTIVE DRY DAYS AT AMES AND CORYDON, IOWA[1]

R.E. Myers and R.H. Shaw[2]

In numerous fields of human endeavor there is a need to know the likelihood of the occurrence of certain types of weather elements. There has been considerable success in the field of weather forecasting in the prognostication of specified elements for periods of short duration, i.e., for one to five days and, in more general terms, the average departure from normal conditions during a thirty-day period. This type of forecast is based on the actual state of the atmosphere at the time the forecast is prepared and upon the changes that are expected to occur according to a well established set of physical laws. The accuracy of forecasts prepared in this manner decreases rapidly as the time of the forecast is extended. When the period becomes sufficiently long or covers a period at some future date this method loses much of its value and we must look to other means of securing the desired information.

Advances are being made in long range forecasting and this problem may be solved at some future time; however, under our present state of knowledge the best method of securing the probability of the occurrence of any weather element during a period at a distant date, appears to be a critical examination of the appropriate climatological data.

The particular problem of determining the probability of consecutive dry days was selected because of the paramount importance that a series of days with little or no rain plays upon a broad segment of the populace. The information concerning the probability of a series of consecutive dry days can be important in two manners that are opposite in nature. First, there may be a task which requires a certain length of rain-free days for its completion and it is desired to know the most logical time to undertake it. Second, from an agricultural point of view there is a high degree of importance connected with periods of drought which extend long enough to be injurious to the crop.

The present study is designed to produce a method of rapidly estimating the probability of the occurrence of a dry period of any given length, where in this case a dry day is considered as a day with less than 0.20 inch of precipitation.

REVIEW OF LITERATURE

There is a considerable amount of information available on the average or normal amount of rainfall, and on the extreme values reached at

[1]Journal Paper No. J-3602 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1280.

[2]Formerly Graduate Student, Agricultural Climatology, now Aviation Forecaster, U. S. Weather Bureau, Denver, Colorado, and Professor, Agricultural Climatology, Agronomy Department, Iowa State University.

various locations, but little has been written concerning the problem of consecutive dry days without interruption by rain. In general, the study of possible drought conditions has been undertaken in the past from the opposite point of view, that is by assessing the probability of receiving a certain amount of rain in a given period of time (1, 5). Blumenstock (2) made an analysis of runs of dry days. A run of dry days was considered terminated by a 48-hour period in which 0.1 inch or more of precipitation was recorded. Because of the very small amount of rainfall used, this study has limited value in the general agricultural field.

Decker (3, 4) has examined the likelihood of extended dry periods in Missouri. In his study he determined the length of runs beginning in semi-monthly periods. He fitted separate curvilinear regression lines to dry periods of less than 20 days and more than 20 days. In some cases this method shows an abrupt break in the accumulated frequencies of dry days at the point between periods of less than 20 days and more than 20 days.

METHODS AND RESULTS

In this study, a dry day was defined as a day with less than 0.20 inch of precipitation. This is an arbitrary limit but does represent the approximate amount of water used by a rapidly transpiring plant on a summer day. It is also the same limit used by Decker (3, 4) in his study in Missouri. A dry period was accordingly defined as the number of consecutive days with less than 0.20 inch of precipitation.

The data for Ames and Corydon which had previously been placed on punched cards were separated into two groups, one with days having 0.20 inch or more of precipitation and the other, those with less than this amount. The cards were coded accordingly and returned to chronological order. The next step was to rerun the IBM cards with the following data being extracted: The day, month and year on which each run of dry days and each run of nondry days began, the number of days in each run and the amount of precipitation, if any, during that run. This material was used to determine the probability of the occurrence of dry periods of various lengths.

In climatological work, it is customary to designate March 1-7 as week No. 1 of the climatological year, with succeeding weeks numbered in order. This practice has been followed, and this numbering system has been used for the different weeks.

The middle day of each week was selected as a representative day for that week. A count was made to determine the frequency of occurrence of dry periods of various lengths in which that day was involved during the years 1901-55. This method did not specify when the run started, only that the day being considered was involved in the run of dry days.

After plotting histograms of the length of runs for individual weeks throughout the year it appeared that the incomplete gamma function would fit these histograms satisfactorily. Over 50 individual weeks for the two locations were tested for goodness of fit by the Chi square method. Only in 5 cases was the fit a statistically poor fit. The average of the others was near the 50% level. This was considered satisfactory and the incomplete gamma function was fitted to each week's data.

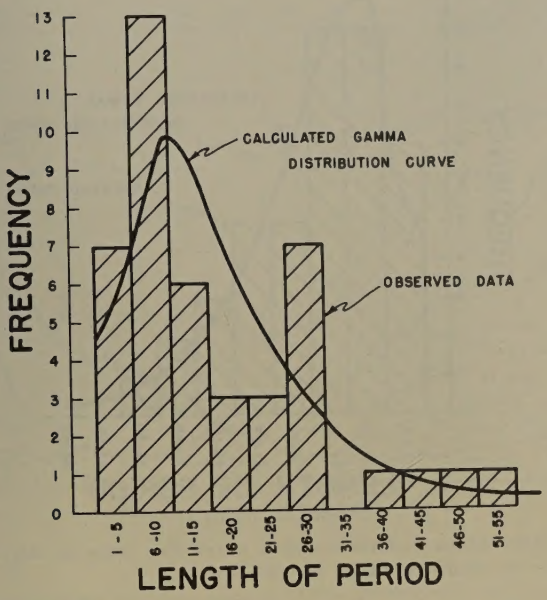

Figure 1. Histogram and theoretical curve for runs of dry days for week number 24 at Ames, Iowa.

Sample theoretical curves and histograms are shown in Figs. 1 and 2. Week 7 represents a good fit, week 24 was one of the poorest fits obtained of the weeks tested. No good physical explanation was developed which explained the bimodal pattern found on some occasions. Investigation of this case points out the ease with which the pattern changes in small samples. The original data were examined and it was found that a one-day change in the length of a portion of the runs for the two peak durations would have brought the histogram into close agreement with the theoretical unimodal curve. A slight shifting of the arbitrary interval used for the length of period could also shift the pattern of the histogram.

The incomplete gamma function was fitted to the observed frequency distributions for each week using the method described by Barger and Thom (1). From these the probability of the occurrence of various dry periods could be determined. There was considerable random variation in the probabilities of runs of different duration, so an arbitrary smoothing of the short time fluctuations was made. The general method of smoothing was to consider a fluctuation based on a single or very few individual values as being erratic and they were adjusted into the general slope of the line for that period. Fluctuations based on a number of weeks were considered to be more valid and any smoothing in these cases was of a minor nature. In Fig. 3 the nonsmoothed observations

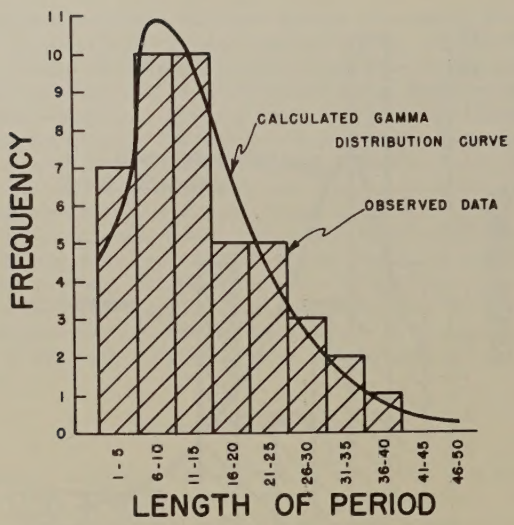

Figure 2. Histogram and theoretical curve for runs of dry days for week number 24 at Ames, Iowa.

are presented for Ames. The curves for 0 and 30 day duration shown in Fig. 4 can be compared with these to see the extent of the smoothing done. In Tables 1 and 2 the smoothed values of the probabilities are presented.

DISCUSSION

The probability of dry days (<0.20 inch of precipitation) is high, and relatively constant throughout the year, or, looking at this from the wet day standpoint, the probability of a wet day ($\geq$0.20 inch of precipitation) is low and relatively constant throughout the year (Fig. 4). An estimate of the confidence band of the probability of runs of different duration can be obtained from the equation

$$s = \sqrt{\frac{pq}{n}} ,$$

where p = probability of run greater than a given duration
$q = 1-p$
n = 55, the number of years of observation.

There was no significant difference between Ames and Corydon, although the probability of a dry day was generally slightly higher for Ames. The probability of a dry day ranged from 79 per cent in early June, to 94 per cent during the winter period. During the growing season there was an

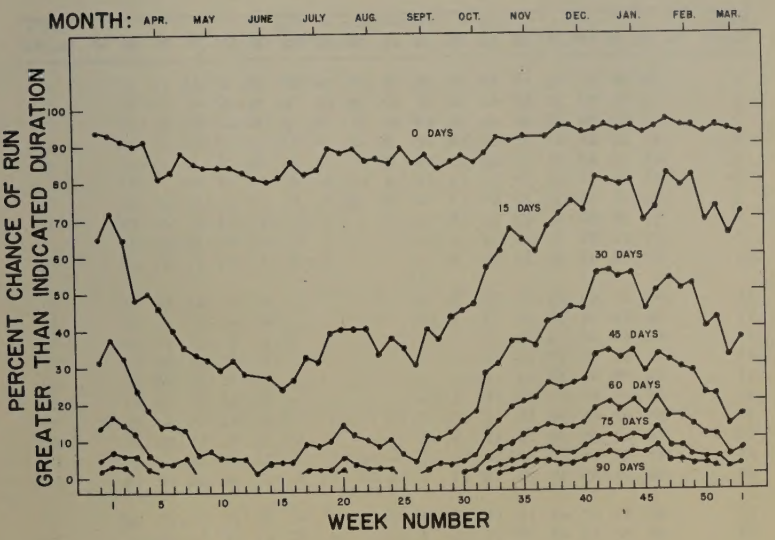

Figure 3. Estimated per cent chance of the occurrence at Ames, Iowa, of runs of dry days of length greater than indicated durations (percentages derived from weekly gamma distribution curves based on date from 1901 through 1955).

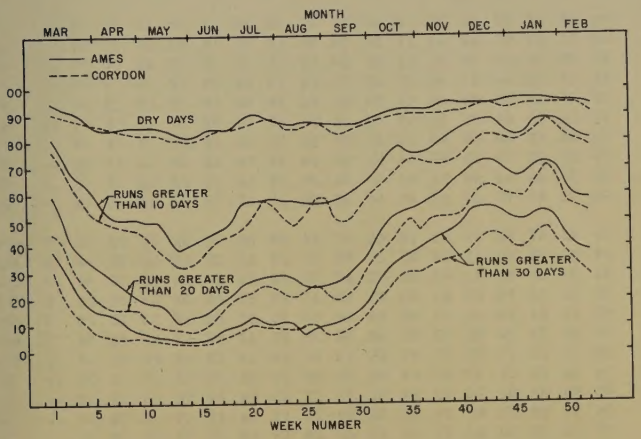

Figure 4. Smoothed curves of per cent chance of occurrence of runs of dry days for Ames and Corydon, Iowa.

Table 1. Probabilities of runs of consecutive dry days at Ames of length greater than the given durations

Week No.	Duration of Run																		
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95
1	94	90	81	70	59	47	38	29	22	17	13	10	7	5	--					
2	92	85	74	61	50	40	32	24	20	14	10	9	7	5	--					
3	91	80	66	52	40	32	24	19	14	11	9	6	5	--						
4	89	78	64	49	35	28	18	12	9	6	--									
5	85	76	60	45	32	24	15	10	7	--										
6	84	74	53	40	29	21	14	10	6	--										
7	84	70	50	35	27	17	13	8	6	5	--									
8	85	70	50	33	23	12	9	5	--											
9	85	70	50	31	20	11	7	--												
10	85	70	49	30	18	11	6	--												
11	85	70	49	29	18	10	5	--												
12	84	69	44	27	15	7	5	--												
13	82	63	38	23	10	--														
14	81	63	40	23	13	6	--													
15	82	64	42	25	13	9	--													
16	84	66	44	27	15	11	5	--												
17	84	67	46	30	19	12	8	5	--											
18	84	69	49	35	21	13	9	5	--											
19	88	72	56	38	25	16	10	6	--											
20	90	75	57	40	27	19	13	9	6	--										
21	89	75	57	40	28	18	11	7	5	--										
22	87	76	58	40	29	17	10	7	--											
23	86	76	57	39	29	16	11	7	--											
24	86	76	57	38	27	16	10	6	--											
25	87	76	56	37	24	11	6	--												
26	87	75	56	37	24	10	9	5	--											
27	86	75	56	37	25	14	10	6	--											
28	86	74	57	38	26	19	11	7	--											
29	86	75	59	41	28	20	12	8	5	--										
30	86	76	62	44	32	23	15	10	8	--										
31	88	78	65	49	36	29	18	13	11	7	5	--								
32	89	81	69	55	44	34	27	20	14	11	9	6	5	--						
33	91	85	75	60	50	40	32	25	19	15	11	8	7	5	--					
34	92	86	78	65	53	44	35	29	23	18	14	10	9	7	5	--				
35	92	85	75	65	54	44	37	30	25	20	16	12	10	8	6	5	--			
36	92	84	73	64	55	44	39	32	26	21	18	14	12	10	8	6	5	--		
37	92	86	76	66	58	48	42	34	28	24	19	16	14	11	9	8	6	--		
38	94	89	80	70	60	53	44	35	30	24	19	16	12	10	8	7	6	--		
39	95	90	83	74	64	55	46	37	30	25	20	16	13	10	8	7	5	--		
40	94	92	85	77	67	56	49	40	32	28	21	17	15	12	9	8	6	--		
41	94	92	87	80	71	63	54	45	37	34	26	20	18	15	10	10	8	5	--	
42	94	92	88	80	72	64	55	46	40	34	28	23	20	16	12	10	8	6	5	--
43	94	92	88	80	72	63	55	46	40	33	28	23	20	16	13	11	9	7	5	--
44	95	91	84	79	69	62	53	45	39	33	27	23	20	16	13	12	10	8	6	5
45	96	89	82	74	64	56	50	43	36	31	26	21	19	16	13	12	10	8	6	5
46	96	89	83	74	66	56	50	44	36	32	27	23	20	17	13	12	10	7	6	5
47	96	94	88	79	71	61	53	45	38	31	26	23	19	16	11	10	8	6	5	--
48	96	94	88	81	72	61	53	44	36	30	24	20	16	13	9	8	6	--		
49	95	93	88	80	70	60	50	41	33	27	21	18	14	10	8	6	5	--		
50	95	91	85	73	62	54	43	36	29	23	18	16	12	9	7	6	5	--		
51	95	90	82	70	58	52	39	32	26	20	16	13	10	8	6	5	--			
52	94	89	81	70	58	49	38	30	23	18	13	11	7	5	--					

Table 2. Probabilities of runs of consecutive dry days at Corydon of length greater than the given durations

Week No.	Duration of Run																			
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95
1	90	87	76	61	45	38	30	20	9	8	6	--								
2	89	83	70	55	40	26	20	14	8	5	--									
3	88	78	62	46	30	20	14	9	6	--										
4	87	76	58	40	26	18	11	6	--											
5	86	72	51	31	20	10	7	--												
6	85	71	49	30	17	9	6	--												
7	84	70	48	30	16	9	5	--												
8	83	69	47	30	16	8	5	--												
9	82	69	46	29	16	8	5	--												
10	82	68	43	21	11	6	--													
11	82	69	38	19	9	--														
12	80	61	35	17	8	--														
13	80	58	32	15	8	--														
14	79	58	32	15	7	--														
15	80	59	35	15	8	--														
16	82	65	40	20	10	--														
17	84	68	43	26	15	7	5	--												
18	84	68	44	29	19	10	6	--												
19	85	68	46	31	21	13	8	5	--											
20	86	69	50	34	22	14	10	6	--											
21	88	78	57	39	25	14	9	5	--											
22	88	79	55	39	24	14	9	5	--											
23	84	70	50	34	21	14	8	5	--											
24	84	68	48	31	20	14	8	5	--											
25	85	70	52	35	23	14	10	6	--											
26	87	78	58	40	25	16	10	5	--											
27	85	77	58	38	21	12	6	--												
28	82	69	49	31	19	11	6	--												
29	83	67	49	31	21	13	8	5	--											
30	85	72	53	36	24	17	10	6	--											
31	86	76	59	45	32	24	15	10	8	--										
32	88	77	62	49	37	28	20	14	10	7	5	--								
33	89	78	65	51	40	30	23	17	12	9	7	5	--							
34	90	85	70	59	46	36	29	20	15	11	9	6	5	--						
35	90	85	73	61	50	40	30	23	16	13	10	8	6	5	--					
36	90	83	72	58	46	40	30	24	18	15	12	9	7	6	--					
37	90	83	71	60	50	40	32	25	20	16	13	10	8	6	5	--				
38	90	84	71	61	51	41	34	27	22	17	14	11	9	7	6	5	--			
39	91	84	72	62	51	42	35	29	23	17	15	11	9	7	6	5	--			
40	91	85	74	62	52	44	36	30	24	18	15	11	9	7	6	5	--			
41	92	89	79	68	59	48	40	33	26	20	17	12	10	8	6	5	--			
42	94	90	82	73	63	52	44	35	28	23	18	14	12	9	7	5	--			
43	94	90	82	73	63	53	45	36	30	25	20	16	13	10	8	6	5	--		
44	92	89	81	70	60	52	43	35	28	23	18	14	12	9	7	6	5	--		
45	93	90	80	70	59	50	39	31	26	20	15	12	9	7	6	5	--			
46	94	90	82	70	59	50	39	31	24	20	15	11	9	7	5	--				
47	94	91	84	75	65	54	45	35	28	22	17	12	10	7	5	--				
48	94	93	88	81	70	58	47	36	28	21	16	12	9	7	5	--				
49	94	92	85	79	66	54	42	32	25	19	15	10	8	6	--					
50	94	90	81	70	56	46	37	29	22	17	13	9	7	5	--					
51	94	90	80	68	55	42	33	25	18	14	10	7	6	5	--					
52	91	89	78	67	53	40	29	21	13	11	7	--								

increase in the probability of dry days from 79 per cent in early June to 90 per cent in mid July, then a slight decrease in August and September. As would be expected the seasonal trends for all lengths of dry day runs were similar to seasonal trends in precipitation.

The length of the run of dry days which will be important to the individual will depend upon what application is to be made of the data. The seasonal trends of runs greater than 0, 10, 20, and 30 days are shown in Fig. 4. The different length runs show similar trends and other length runs would be expected to have the same pattern.

There is a relatively high expectancy of dry periods during much of the winter period, with a rapid decrease in the expectancy starting in early February, and a general decline continuing until late May or early June, when the probability of runs of dry days reaches a minimum. Corydon shows a sharp decrease in late April, then a gradual decline. The likelihood of dry periods rises during June, with the likelihood relatively constant during July and August, although Corydon showed a decrease in early July and an increase in late July for 10 and 20 day periods. In early September the likelihood of dry periods increases at Ames and continues to increase into the winter period. At Corydon, there was a sharp decrease in early September, then a gradual increase into the winter period.

A careful examination of the probabilities shows a slight time displacement in many cases, between Ames and Corydon. The likelihood of runs of dry days is generally less at Corydon than at Ames, although the same seasonal trends are evident. The largest differences between Ames and Corydon is for a run greater than 10 days in early April, when the likelihood at Ames is 18 per cent greater. Over the year, Corydon will average about 5 per cent less likelihood of a run of dry days than Ames.

In the future it is intended to extend this analysis to at least two other areas in the state, the southeast, the wettest area, and the northwest, the driest area. The northeast part of the state should probably also be examined in this analysis. With these additional areas, a better evaluation of the runs of dry days can be made, particularly the difference in likelihood across the state and any change in time phase across the state.

This method of analysis does not answer the question, "What are the chances of a run of dry days of a given length after today?" Taking any day throughout the year, the probability of a run of dry days of a given length after that date is considerably smaller than half the probability of a day being involved in a run of dry days as was done in this study. It would require a different type analysis to answer this question.

SUMMARY

A dry day was defined as a day with less than 0.20 inch of precipitation. Using IBM punched cards, the runs of dry days were tabulated for Ames and Corydon. The frequency distribution of the length of run in which the middle day of each climatological week was involved were computed, and the incomplete gamma distribution fitted to this distribution. The probability of runs of consecutive dry days was determined for each week from these curves.

The probability of a dry day was relatively constant throughout the year, although the likelihood was lowest in the late spring and highest in the winter. The probability of runs greater than 5, 10, 15, etc. days showed a pronounced seasonal pattern, with the least likelihood of a run in the late spring and the highest likelihood in the winter. The likelihood at Ames was generally slightly higher than at Corydon.

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SOME ASPECTS OF THE LIFE HISTORY OF THE CARP, CYPRINUS CARPIO, IN THE DES MOINES RIVER, BOONE COUNTY, IOWA[1]

Dudley Dean Rehder[2]

Department of Zoology and Entomology
Iowa State University, Ames

Abstract

Carp were collected, mostly with an electric shocker, from a 7-mile section of the Des Moines River in 1958 and the fall of 1957. Weight was found to increase as the 3.02456 power of the total length, but the difference from the cube relationship was not statistically significant. Condition factor, C, therefore, showed no trend with length of the fish. Length-weight relationships of male and female carp were similar. Condition factors were highest in midsummer, at a time of high water level. On the average, standard lengths equal 0.798 total lengths and fork lengths equal 0.890 total lengths, with conversion factors being somewhat higher as length of the fish increases. Scales and opercles were used in determining age and growth rates of the carp. Age determinations from these two structures were in agreement in 84.5% of the fish. Resumption of growth was evident on the opercle earlier in the spring then on scales. The body-opercle relationship was more nearly rectilinear and showed a better correlation than the body-scale relationship. Growth of carp in the Des Moines River appears to be about average in comparison to published reports. A few of each sex mature at age II. Presence of two sizes of eggs in the ovaries of some females indicated a possible spread of the spawning season. Number of eggs per female increased with age and size. Age IV females averaged 181,000 eggs. The most abundant year class, 1956, was produced during a year of low stable water levels. During the summer of 1958 carp fed mostly on seeds (22.3%), other plant material (54.8%), and insects (10.3%). Earthworms and terrestrial insects comprised most of the food during floods. An unsegmented tapeworm tentatively identified as Caryophyllaeus laticeps was found in many carp. Although a common parasite of carp in Europe, it has not been previously reported in America.

[1] Journal Paper No. J-3606 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1373. Iowa Cooperative Fisheries Research Unit sponsored by the Iowa State Conservation Commission and Iowa State University, with the cooperation of the Bureau of Sport Fisheries and Wildlife, U.S. Department of Interior.

[2] Now Naturalist for Corps of U.S. Engineers, Fort Randall Dam and Reservoir, Pickstown, South Dakota.

Introduction

Although the carp is one of the most important fish caught by anglers in the Des Moines and other Iowa rivers, little is known about its rate of growth or other life history in these waters. The specimens for the present study were collected from October 1957 to October 1958 in a 7-mile stretch of the Des Moines River from the Fraser Dam to the Boone Waterworks Dam. Most of the carp were taken with an electric shocker.

Length-Weight Relationship

Total lengths (tip of snout to end of caudal fin with the lobes squeezed together) are used throughout this report but standard lengths (to crease depicting end of hypural plate) and fork lengths (to center of fork of caudal fin) were measured on most fish. Factors for converting total to fork or standard lengths increase with increase in length of fish (Table 1).

Table 1. Length conversion factors for Des Moines River carp, 1957 and 1958, arranged by 4-inch size groups

Total length	Number of specimens[a]	Total to fork length	Total to standard length
7-10	31	0.876	0.788
11-14	40	0.885	0.793
15-18	18	0.892	0.804
19-22	35	0.892	0.798
23-26	13	0.896	0.805
27-30	6	0.907	0.803
Total	143		
Average		0.890	0.798

[a]10 fish whenever possible, were arbitrarily selected from each 1-inch group for determining conversion factor.

It has been established that the length-weight relationship of fish usually may be expressed by the formula:

$$W = cL^n$$

where W = weight, L = length, c and n = constants

When the weights and lengths are transformed to logarithms, the relationship is linear:

$$W = \text{Log } c + n \text{ Log } L.$$

Table 2. Mean lengths and mean weights of carp from Des Moines River study area. Arranged in 1-inch groups

Female			Male		
Mean total length	Number of specimens	Mean weight (lbs.)	Mean total length	Number of specimens	Mean weight (lbs.)
7.4	1	0.13	--	0	--
8.6	16	0.32	8.7	11	0.33
9.5	34	0.45	9.4	38	0.42
10.4	52	0.57	10.4	59	0.57
11.5	55	0.74	11.4	54	0.76
12.3	42	0.91	12.2	17	0.90
13.4	18	1.20	13.3	13	1.16
14.4	9	1.41	14.1	4	1.37
15.7	1	2.13	15.5	1	1.75
16.2	1	2.19	--	0	--
17.3	3	2.46	17.4	2	2.50
18.3	6	3.02	18.6	7	2.99
19.5	11	3.69	19.3	7	3.33
20.6	5	4.21	20.6	2	4.12
21.5	6	4.82	21.6	3	4.94
22.6	4	5.67	22.7	2	5.25
23.4	1	7.00	23.3	2	6.18
24.6	2	6.71	--	0	--
25.1	2	7.34	25.0	1	7.31
26.5	3	9.58	--	0	--
27.1	3	9.50	--	0	--
28.0	2	11.15	--	0	--
--	0	--	--	0	--
30.7	1	14.37	--	0	--

The average weights of male and female carp at 1-inch length groups appear to be quite similar (Table 2). An analysis of covariance indicates that differences in the regression lines and in the adjusted mean weights of the males and females are not sufficient to be considered significant (Table 3). Therefore a single equation was computed:

$$\text{Log } W = -1.34370 + 3.02456 \text{ Log } L.$$

The use of average weights and lengths by inch classes in the analyses of covariance is not entirely valid. Since this short cut method underestimates the within sample variance, nonsignificant F values determined from these variances can be assumed to be nonsignificant. Confidence

Table 3. Analysis of covariance and test of significance of the hypothesis that the regression coefficient, b, is the same in the length-weight regressions of male and female carp * and that the adjusted mean weights are the same.

Source of variation	Degrees of freedom	Sums of squares and products Σx^2	Σxy	Σy^2	Reg. coef. b
Female	22	0.69527	2.13960	6.62589	3.077
Male	15	0.33046	0.96279	2.80678	2.913
Common	37	1.02573	3.10239	9.43267	3.024
Adjusted means	1	0.01518	0.04700	0.15123	
Total	38	1.04091	3.14939	9.58390	

Source of variation	Degrees of freedom	Deviation from regression $\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	Mean square
Female	21	0.04156	0.00197
Male	14	0.00177	0.00012
Within	35	0.04333	0.00209
Reg. coef.	1	0.00596	0.00596
Common	36	0.04929	0.00136
Adjusted means	1	0.00581	0.00581
Total	37	0.05510	

Test of regression coefficients $F = 0.00596/.00209 = 2.85$

Test of adjusted means $F = 0.00581/.00136 = 4.27$

* The system of notation in these analyses is similar to that outlined in table 13.2.2 of Snedecor (1956). Σx^2 indicates the sum of the squares of the deviations from the mean length. Σxy indicates the sum of the products of the deviations from the mean length and mean weight. Σy^2 indicates the sum of the squares of the deviations from the mean weight. F is the variance ratio, the significance of which is evaluated according to the distribution discovered by R. A. Fisher, as given by Snedecor (1956).

Table 4. Mean condition factors of the Des Moines River carp in various months.

Date	Number of carp	Mean C
1957		
October	30	49.2
1958		
April	9	46.6
May	27	54.0
June	46	49.0
July	169	51.0
August	218	48.7
September	6	47.0
October	12	45.7
Combined	517	49.6

limits at the 95 per cent level indicate that the population regression coefficient will fall within the range of 2.96 and 3.08 unless the sample is one of the divergent kind which may occur about once in 20 trials. Since the regression coefficient does not differ significantly from 3.0, the condition factor, C, should show no general trend with increase in length of the carp, when

$$C = \frac{W10^5}{L^3}$$

W = weight in pounds and L = total length in inches.

Inspection of C values showed no trend with length. The C values (Table 4) increased rapidly during the spring with the resumption of growth, dropped during late spring and early summer possibly correlated with spawning activities, reached a peak during midsummer and then fell off through late summer and fall. During the month of July when C values were high, frequent rains causing high water levels were common. During this period of high turbid water much of the river flood plain was inundated providing additional habitat for the fish. The Des Moines River carp appear to have average condition factors in comparison to carp from other areas (Carlander, 1953, p. 324).

Age and Growth

Scales and opercular bones were examined to determine the age and growth of fish during this study. Scale samples were taken from an area on the left side of the fish three rows above the lateral line and directly below and forward of the dorsal fin. Before removing the scales from the fish the area was scraped clean of slime and foreign material. The scales were prepared for projection utilizing the plastic impression

method as described by Smith (1954). Readings were made from the projected image of scales using a projection apparatus similar to that described by Van Oosten, Deason and Jobes (1934). All scales were examined at a magnification of 17 diameters.

Annuli on the scales of yearling carp were quite distinct and little difficulty was encountered in determining their age. Growth during the first summer showed a different spacing of the circuli compared with those of ensuing summers. This same characteristic was described by English (1952).

The scales of II-year-old carp show cutting-over in the lateral field at the point of the first and second annuli. The age of the II-year-old carp was established with little difficulty. Carp III-years and older were aged similarly. However, with an increase in age an increase in difficulty in interpreting the age was encountered.

Opercles were collected about as described by English (1951) and McConnell (1952). Opercles were placed in boiling water for about 1 minute and then scoured with a stiff bristled brush. They were thoroughly dried before examination. Annuli appear as distinct, narrow bands parallel to the posterior margin of the opercular bone. The area enclosed by each annulus is of the same shape as the opercular bone. As suggested by McConnell (1952) this characteristic indicates that the annulus was the margin of the opercular bone at some earlier time in the life of the carp. Each annulus is composed of two parts. Posteriorly the annulus is a thin band which appears darker than the surrounding area. The second area which lies within this band is laid down during the period of active growth and appears wider and is more translucent.

The opercles were examined from both the concave and convex surfaces in reflected light. Examination of the opercular bone from the convex side was required in locating the first annulus on many of the larger opercular bones. The buttresses of bone growing out from the opercular socket on the concave side often covered that area where the first year mark would normally appear making it difficult to locate from an examination of the concave side. An examination of the convex side was also helpful in distinguishing true annuli from false as the true annuli appear more distinct on the convex side than the false annuli.

Resumption of growth was evident on opercular bones earlier in the season than on scales. The first annuli on scales were observed May 17.

Two independent readings were made of each scale and each opercle without reference either to fish length or previously estimated age. Adjustments of age disagreements with each respective method were made, when required, by means of a third reading with the knowledge of the fish length, capture date, and previously ascribed ages. The final age determinations of the scale and opercle were then compared and disagreements were resolved, when possible, by a joint examination of the scale and opercle.

Of the 520 carp collected, 52, or 10 per cent, could not be aged with the scale method because scales were abnormal or regenerative. In two cases both scales and opercles were discarded from the same fish.

The majority of the carp could be aged by the opercle method with little difficulty. Of the 520 carp collected during this study, 10, or 1.9 per cent, could not be aged with the opercle because of damaged or deformed structures. Thus, a total of 510 carp were aged with this method.

The final age determination of those fish aged with both the opercle and scale were in agreement for 376 fish or 84.5 per cent. Only one disagreement differed by more than one year. The fish for which scale and opercle readings differed ranged from 8.9 to 30.7 inches in total length and from 1 to 9 years in age. McConnell (1952) in his studies of the carp found 66 per cent of the scales that could be read gave the same age as the corresponding opercular bone and 91 per cent agreed within one year.

Of 74 sets of disagreeing readings 57 were resolved by the joint reading; and 17 sets remained in permanent disagreement. Of the 57 sets of resolved readings, 13 scale determinations were adjusted to younger ages and 28 to older ages, making a total of 41 adjustments. Some of the adjustments to older ages were of fish captured in the spring when annulus formation was not distinguishable on the scale but was considered evident on the opercle. The opercle readings were adjusted to younger ages in seven sets of readings and to older ages in seven cases, making a total of 14. Readings of both structures were adjusted in two cases.

In addition to age, the past growth history of a fish can be determined from scale measurements if the relationship between scale and body growth is known. The relationship between the total length in inches and the anterior scale radius in millimeters (X17) was determined from a subsample of 10 fish (whenever possible) from each inch group. The 10 fish from each inch group were selected in accordance with the random numbers method as described by Snedecor (1956). A regression line having a Y-intercept (length axis) of -0.1712 inches and slope of 0.1038 was fitted to the subsampled data by the least squares method (Fig. 1).

The points representing mean scale radii of the carp at 1-inch intervals (all the carp, not just the selected sample) suggest a sigmoid body-scale relationship rather than a straight line. That the straight line fits quite well is indicated by the regression coefficient, r, of 0.972.

Measurement of growth to each annulus on the opercles was made from the posterior margin of the fulcrum to the most posterior point of the annulus. McConnell (1952) and Le Cren (1947) have both demonstrated by cross sections through the fulcrum that the posterior margin of this structure is directly over the origin of growth of the posterior field of the opercular bone. The annuli of each opercle were outlined in pencil on the concave surface. The distance from the fulcrum to the outlined annuli and to the posterior edge of the opercle were then measured directly with a transparent millimeter rule. A small notch cut in the rule at the zero point helped to hold the rule at the zero point on the fulcrum edge.

The same subsample used in determining the body-scale relationship was used in computing the body-opercle relationship. A regression line having a Y-intercept (length axis) of -0.6980 inches and a slope of 0.5705 was fitted by the method of least squares (Fig. 2). The body-opercle relationship appears to be more nearly a straight line than the body-scale relationship. The regression coefficient, r, is 0.998.

Since the calculated body-scale and body-opercle lines intercept the length at a point near zero, growth computations were made using zero as base on a nomograph (Carlander and Smith 1944). Lengths calculated

Table 5. Average calculated total lengths at each annulus as computed from opercles (O) and from scales (S) from carp collected from the Des Moines River, Boone County, 1958.

Year class	Age group	Number examined		Average total length in inches at each annulus 1	2	3	4	5	6	7	Total length at capture Mean	Range
1958	0	1									3.3	
1957	I	11	O	7.1							9.7	8.4-11.5
			S	6.2								
1956	II	44	O	6.4	9.7						11.1	8.2-14.4
		339	S	5.0	9.2						10.9	8.2-14.4
1955	III	30	O	6.0	10.4	14.3					15.8	11.2-21.0
			S	4.7	9.5	14.2						
1954	IV	17	O	7.2	11.4	15.7	18.8				19.8	17.0-22.9
			S	5.3	9.9	14.3	18.5					
1953	V	7	O	7.6	10.5	13.7	17.3	19.4			20.5	17.2-24.7
			S	5 1	9.7	12.8	15.9	19.0				
1952	VI	5	O	7.4	12.1	16.9	20.4	23.0	24.4		25.1	23.2-27.0
			S	6.9	11.2	16.0	19.4	22.5	24.2			
1951	VII	3	O	8.2	12.3	15.9	19.6	21.0	23.0	24.3	25.0	22.9-27.3
			S	5.5	10.9	14.9	18.0	20.0	22.5	24.0		
Total		117	O	6.6	10.4	14.9	18.8	20.9	23.8	24.3		
		413	S	5.0	9.3	14.2	18.0	20.4	23.6	24.0		
Annual increment		117	O	6.6	3.8	4.0	3.3	2.1	1.6	1.3		
			S	5.0	4.3	4.4	3.7	2.9	2.0	1.5		
Average weight *		413	S	0.06	0.38	1.39	2.83	4.14	6.44	6.77		

* Weights are given in pounds, calculated from the length-weight equation Log W = -1.3437 + 3.02456 Log L.

from opercular measurements were somewhat higher than those calculated from scale lengths (Table 5). Part of this difference may be the result of the 0-0 intercept used in back calculating on the nomograph. The a intercept of the body-opercle relationship (-0.698 inches) was farther from the zero point than that of the body-scale relationship (-0.171 inches). Correction for this intercept would reduce the opercle-calculated lengths by somewhat less than one-half inch. The remaining differences are probably due primarily to differences in determining the true point of annulus formation. Although it was not realized during the study, a bias in measuring the opercular year marks to their maximum distance and the scale year marks to some place inside the maximum distance might have occurred.

The growth of Des Moines River carp, as shown by the scales is somewhat below the average reported in other scale studies (Carlander, 1953, pp. 102, 325, 326). Growth of the Des Moines River carp was similar to that found by Shields (1957) in the studies of the carp in Fort Randall Reservoir in South Dakota. When compared to the growth rate of Salt River, Missouri, carp (Purkett, 1958), the Des Moines River carp show slower growth through the first three years of life, but exceed the calculated growth of these carp in all following years.

Age group II, which comprised 82.6 per cent of the total number of fish in the sample, shows slower growth than the other year classes.

If the annual increments for the different age groups are analyzed according to growth in various calendar years, 1952 seems to have been best. However, growth calculations for this year are represented by only two year classes. During the years 1954, 1956 and 1957 growth could be considered to be above average. The year 1956 was reported to have been a good growth year for channel catfish (Muncy, 1957) and for river carpsucker (Buchholz, 1957) in the same section of the Des Moines River.

Reproduction

The first indication of a spring migrational movement of carp to more desirable spawning grounds occurred on May 14. On this date carp were observed jumping over the lowhead dams and moving upstream. Surface water temperatures had risen steadily during the warm spring to 66°F on May 13. Gonadal condition of seven mature carp captured on May 17 resulted in classification of one male as ripe, two males and one female as near ripe and one male and two females as green. The capture of carp became increasingly difficult from this date until June 25. During this period there was a pronounced increase in water level, stream velocity and turbidity.

The first fish classified as spent were captured on June 25. Presence of ripe, partially spent and spent fish throughout the remainder of the summer suggests that the spawning of carp was of an erratic fashion and that some fish may have failed to spawn. Some ovaries contained atretic eggs and eggs in two distinct levels of development which suggests a possible split spawning season for some females. Shields (1956) reports a prolonged spawning season for carp in Gavins Point Reservoir, South Dakota.

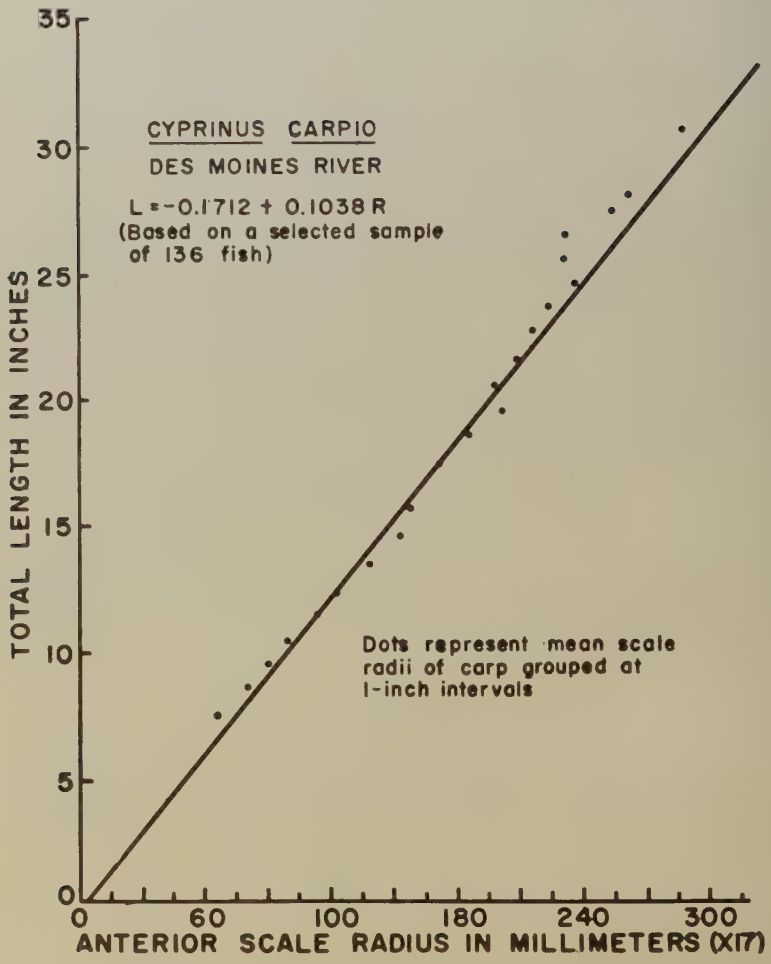

Figure 1. Body-scale relationship for Des Moines River carp from 1957 and 1958 collections.

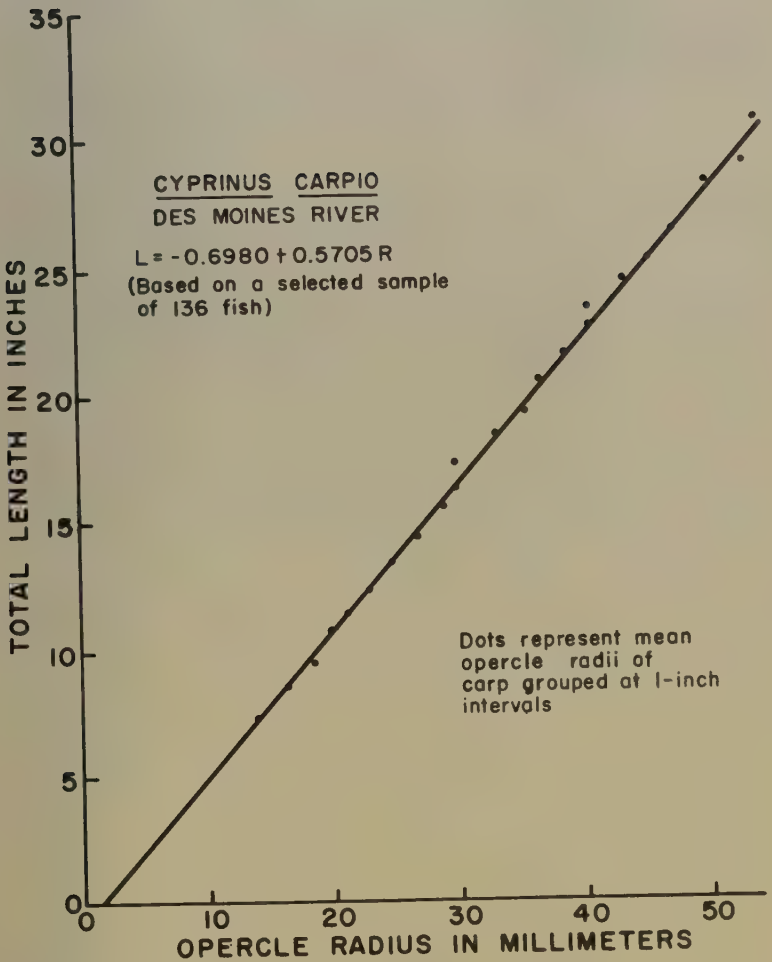

Figure 2. Body-opercle relationship for Des Moines River carp from 1957 and 1958 collections.

Table 6. Comparison of Des Moines River 1955-56-57-58 average monthly water level stages and 15 year average (1933-47 inclusive) with carp year class abundance. All carp were collected during 1958.

Year	Carp abundance	Water gauge reading in feet May	June	July	August	September
15 yr. average[a]	32[b]	2.1	2.8	1.4	1.1	1.2
1955[a]	30	1.1	1.0	0.85	0.25	0.20
1956[a]	339	0.46	0.42	0.40	0.40	0.27
1957	13	0.59	1.29	0.78	0.45	1.41
1958	1	0.65	1.29	1.41	0.41	0.24

[a]Water level data were presented by Muncy (1957).

[b]Includes carp from 1951 to 1954 year classes.

The 1956 year class was obviously the most abundant during the period of study. It is evident the water level readings (Table 6) during the summer of 1956 were consistently lower than any of the other recorded readings and do not show as much fluctuation.

Of the 520 carp collected during this investigation sex was determined for 500 specimens. Males comprised 44.5 per cent (224) of the number collected and females 55.2 per cent (276). Sex could not be determined in 12 II-year-old fish which ranged from 8.5 to 11.2 inches in total length. Of the 11 fish aged as I-year-old, 6 were females and 5 were males. The majority of the II-year-old fish captured were considered to be immature. The largest immature female captured was 13.2 inches in length. Immature males as large as 12.3 inches in length and mature males as small as 9.3 inches were captured during 1958.

The number of eggs per female was estimated by determining the volume of the ovaries by water displacement and then counting the number of eggs in small samples. The number of eggs increases with the age and length of the female (Table 7).

Table 7. Estimated number of eggs per female carp, Des Moines River, 1958

Age group	Number examined	Total length in inches	Weight in pounds	Thousands of eggs per female Mean	Range
III	3	13.6-18.0	1.37-3.06	101	39-137
IV	5	17.0-21.5	2.37-5.00	181	138-215
VI	1	26.5	9.75	335	-

Food Habits

The variety of food items found in this study (Table 8), and in other studies (Adams, 1928; Harrison, 1950; and Moen, 1953), indicates the carp may certainly be considered omnivorous. Harrison (1950) reports the carp diet in the Des Moines River to be 53 per cent insect, 32 per cent plant material, 9 per cent invertebrates other than insects, 4 per cent organic material and 1 per cent fish.

Table 8. Percentage of total volume of food groups of 75 carp by dates and water level, Des Moines River, Iowa (based upon laboratory examination)

Date of collection	Water level in feet	Number of fish	Insect	Plant material	Seeds	Miscellaneous
June 30	0.72F[a]	5	4.6	71.8	23.0	0.4
July 9-14	0.92F-1.28R[b]	25	6.1	59.7	32.5	1.6
July 16[c]	6.12R	7	9.8	13.3	3.6	73.3
July 18	2.58F	7	1.7	52.4	45.8	0
July 30	0.80F	6	1.3	59.8	25.5	13.3
August 4-7	0.56F-0.49F	10	1.4	67.0	15.2	16.3
August 13	0.38F	8	25.3	49.8	11.7	13.2
August 28	0.30F	7	47.3	46.2	2.2	4.2
Combined		75	10.3	54.8	22.3	12.5

[a]F = Falling water level.

[b]R = Rising water level.

[c]Fish captured in inundated-flood-plain water.

Plant material, mostly green fragments, dead plant material and roots, was the most consistent food type found in carp enterons and made up the bulk of the diet. Seeds of plants also appeared in the diet of carp throughout the season, but the volume and occurrence was greatest during periods of higher water levels probably resulting from heavy rains washing this food into the river. Some of the more common types of seeds appearing in the diet of the Des Moines River carp were: barnyard grass (Echinochloa crusgalli), sweet clover (Melilotus albus), alsike clover (Trifolium hybridum), sedge (Carex sp.), common smartweed (Polygonum persicaria), smartweed (P. sp.), Pennsylvania smartweed (P. pennsylvanicum), Japanese brome (Bromus japonicus), Kentucky bluegrass (Poa pratensis), cowcockle (Saponaria vaccaria), blackberry or raspberry (Rubus sp.), and beggar-ticks (Bidens frondosa).

Insects comprised a relatively small amount of the food until August 13 and 28 when the water levels were fairly stable or falling slowly. Most of the insects were larval midges of the family Chironomidae, usually associated with varying amounts of plant material. Larval ceratopogonid midges and phantom midges (Chaoborus sp.) were taken in small numbers. Immature forms of the mayfly, dragonfly, dobson fly, and caddicefly appeared most frequently during the lower water levels but never in large numbers. Ants and bryozoans (Plumatella repens L.) occurred in the diet of the carp only during late summer at lower water levels. Spiders and terrestrial beetles occurred in greatest numbers immediately following a high water level. Earthworms contributed heavily in the diet of those fish captured in the waters of an inundated flood plain and not at other times. This suggests that carp prefer animal life over plant life when it is available as both food types were in abundance in this flooded area. Moen (1953) reports the diet of the carp, under normal lake conditions, to be predominately animal material.

Several additional intestinal tracts were observed in the field, but they added little to that reported above from laboratory analysis. Two unidentified fish were found in one of the carp examined in the field.

Parasites

In obtaining stomach contents and determining the sex of the carp collected, it was possible to observe the internal organs and record the presence of any parasites or abnormalities which would indicate parasitic infection. Although it is quite probable some parasitic forms escaped detection, it is believed these forms were too limited in number to affect the population as a whole.

A parasite commonly found in the intestine of many carp was a non-segmented tapeworm, tentatively identified as Caryophyllaeus laticeps Pallas, 1781 (Figure 3). In all intestines in which this parasite was found it was present in the upper 1/3 of the intestine and never in any great number. Most fish harboring the parasite contained from two to six specimens and the greatest number observed was eight. English (1952) reported parasites of the family Caryophyllaeidae in the digestive tracts of carp from Clear Lake, Iowa, but no reports of C. laticeps in North America are known to the author. C. laticeps is listed as a common parasite of carp in European waters.

Two parasitic copepods were also commonly found in the Des Moines River carp. The fish louse, Argulus sp., was found on several carp, but never in any numbers. This parasite may have actually been overlooked on several fish as it was difficult readily to detect its presence. The other parasitic copepod which was found in limited numbers was the anchor parasite, Lernaea sp., which was found attached to the fins and to the unscaled body regions.

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The author wishes to express his gratitude to Dr. Kenneth D. Carlander, Professor, Department of Zoology and Entomology, for his assistance and guidance in interpreting the data and preparing this paper;

Figure 3. A parasitic nonsegmented tapeworm, tentatively identified as Caryophyllaeus laticeps, which was commonly found in the digestive tract of Des Moines River carp.

to Dr. L.E. Everson of the Seed Laboratory for identification of certain food items; to Fred Meyer for identification of the parasites; to James C. Schmulbach for assistance in the field; to the Y.M.C.A. Camp near Boone for use of their facilities; and to his wife, Shirley, for encouragement and assistance throughout the study.

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EFFECT OF SANITATION, PACKAGING AND ANTIBIOTICS ON THE MICROBIAL SPOILAGE OF COMMERCIALLY PROCESSED POULTRY[1]

John C. Ayres

Food Processing Laboratory
Iowa State College, Ames

The development of microorganisms is largely responsible for the spoilage of poultry meat. The researches reported here include results on incidence of bacteria and other microorganisms that most frequently spoil fresh meat, species generally associated with defects, general plant procedures influencing bacterial populations, and treatments enhancing the storage life of fresh poultry meats.

Several methods have been tested for estimating numbers of organisms on the skin and lean surfaces of poultry. Among these are: the pressing of metal dishes filled with solidified agar (spot plates) against the skin, the swabbing of known surface areas, the use of cut sections of tissue sliced to known depths, the smearing of material from the underwing area on glass slides, and the rinsing of birds of uniform size and equivalent amounts of water. For reasons of expediency or reliability, advocates of each of these procedures claim its superiority to other methods. For example, Gunderson and associates (15, 16, 18) used a metal "spot plate" to determine the status of contamination on commercially eviscerated birds. The degree of infection was given a rating of I to VI after comparison with a selected standard. Ziegler, Spencer and Stadelman (41) developed a rapid microscopic method for evaluating bacterial populations. This consisted of obtaining material from under the wing and smearing uniformly on a glass slide, staining and examining microscopically. These workers reported that observation of slime smear slides prepared in this manner revealed spoilage earlier than was possible by sensory methods. Mallmann, et al. (21) claimed that shake rinsing provides the most satisfactory index of the numbers of organisms on the bird. They sampled half sections of fryers ranging in weight from 404 to 682 grams in amounts of water equal to 1 ml per gram of weight; the birds were placed in a gallon glass container and shaken mechanically for 2 minutes. In their experience, results with the swab sampling methods were too variable. After comparing rinse, cut-section and swab sampling methods (Fig. 1), workers at Iowa State College (4) selected a moist swab procedure because of its ease of use, economy of materials and rapidity with which it could be used "on the line" in the processing plant. They cautioned that it was necessary to adopt a standardized technique in swabbing the surface and that a rough mopping with a dry swab may have been responsible for some of the variation others had obtained in their results.

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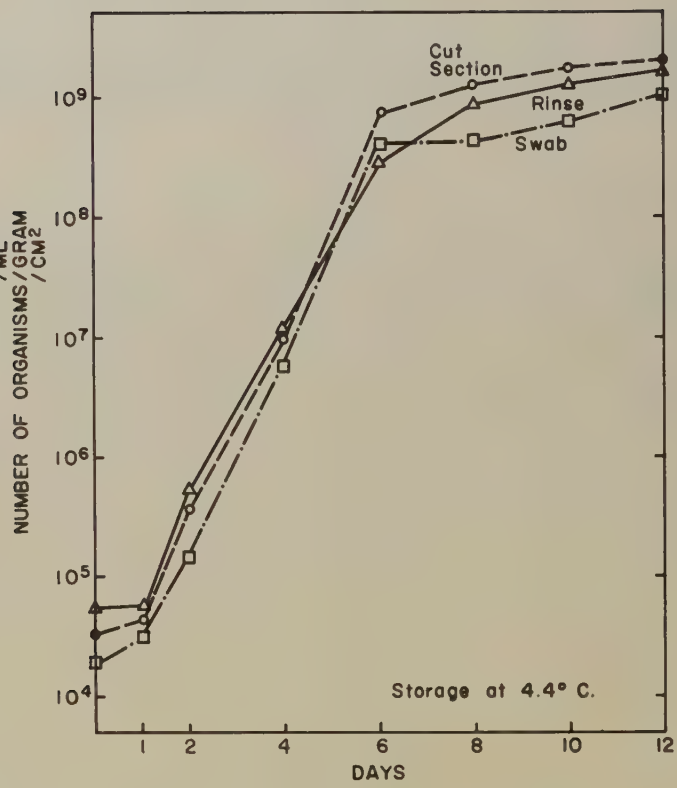

Figure 1. Comparison of growth curves obtained by swab, rinse and cut section methods of sampling.

Another procedure and one that has been widely used for determining the quality of milk, the resazurin reduction test, was studied (4) to find if it could be used for determining the bacterial quality of poultry. For this test, swab samples were placed in peptone water and, after adding resazurin, trypticase soy broth, and skim milk, were incubated at 15° or 30°C until a fluorescent pink color developed. Good correlation was obtained between reduction time and numbers of organisms present on chickens (Fig. 2).

There is need for proper evaluation of the advantages and limitations of various sampling methods for determining the microbiological quality of dressed poultry. An attempt should be made to find the most desirable procedures for research and for plant use; some of the procedures listed above, such as the cut-section- and rinse-methods, do not lend themselves to routine use.

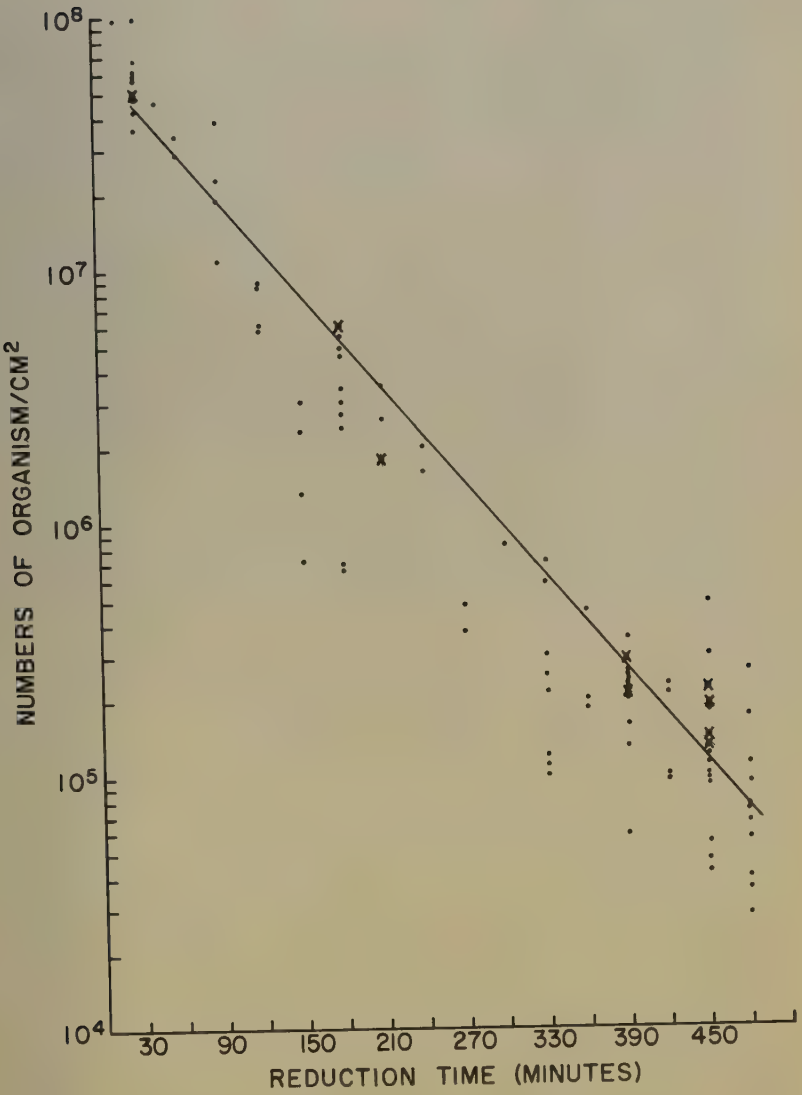

Figure 2. Relation between numbers of organisms and time required to reduce resazurin to a fluorescent pink end point.

Samplings (32) from six poultry processing plants in Iowa indicated a mean population of 1500 organisms per cm^2 on the skin of live birds; approximately 35,000 per cm^2 on the surface or skin of birds immediately after processing. Gunderson, et al. (16) sampled commercially processed poultry in the Omaha area and found that the average initial population for freshly killed, warm eviscerated birds was 4800 per cm^2 while that of birds chilled on ice averaged 60,000 per cm^2. McVicker, et al. (22) reported much lower loads; birds that they sampled were found to have approximately 1,000 organisms per $inch^2$ immediately after processing.

Any of a number of microbial species are likely to be recovered from chicken (2, 17, 20) meat immediately after the bird has been killed and processed (Table 1).

Table 1. Genera of microorganisms isolated from eviscerated poultry

Genus	Investigators		
	Lochhead and Landerkin (20)	Gunderson, Ross and Henn (17)	Ayres, Ovilvy and Stewart (2)
Bacteria			
Pseudomonas		+	+
Micrococcus	+	+	+
Achromobacter	+	+	+
Flavobacterium	+	+	+
Alcaligenes		+	+
Proteus		+	+
Bacillus		+	+
Sarcina		+	+
Streptococcus			+
Eberthella		+	+
Salmonella			+
Escherichia		+	+
Aerobacter		+	+
Streptomyces		+	+
Paracolobactrum		+	+
Staphylococcus		+	+
Corynebacterium		+	
Actinomyces		+	
Hemophilus		+	
Neisseria		+	
Gaffkya		+	
Microbacterium (including "diphtheroids")		+	
Fungi			
Penicillium			+
Oospora		+	+
Cryptococcus			+
Rhodotorula			+
Torula		+	

Chromogenic bacteria accounted for at least half of the total population. Psuedomonas and nonpigmented cocci comprised 1/5 to 1/4 of the flora while the remaining organisms consisted of yeasts and miscellaneous bacteria (Fig. 3). The proportion of chromogens and miscellaneous organisms decreased during storage. Ziegler and Stadelman (42) also studied the microflora on poultry and found the predominant types of organisms on fresh birds to be Gram-negative rods (Pseudomonas, Flavobacterium) and yeasts. In another study, Wells et al. (38) reported that at 9° and 12°C over 50% of the colonies were similar to Flavobacterium.

Within a few days after processing a rather uniform psychrophilic flora predominates and causes the development of off odor and slime on the product (Fig. 4). Both indications of deterioration were found by Ayres, Ogilvy and Stewart (2) to be closely associated with the growth and coalescence of colonies of several species of Pseudomonas. These organisms reproduced rapidly. At the time of incipient spoilage more than 99 per cent of the total bacteria flora were species of Pseudomonas and Gram-negative cocci. A population of about 100 million organisms per cm^2 was considered sufficient for visual manifestations of slime. Relationship of several of the strains of pseudomonads recovered to species characterized in Bergey's Manual, 6th Ed. (7) was shown. The importance of the Gram-negative cocci was not determined but was considered of minor importance.

It should be noted that on birds treated with chlortetracycline or oxytetracycline, yeasts comprised a significant proportion of the population (Fig. 5). Wells and Stadelman (39) found yeasts isolated from treated and untreated birds to be made up of representatives of the genera Rhodotorula, Torulopsis and Cryptococcus. The proportions and percentages of various organisms were related somewhat to the temperature of storage. Njoku-Obi et al. (25) found Rhodotorula and Torulopsis. They also found species of Saccharomyces, Candida, Geotrichum and several genera of molds. Both pigmented and nonpigmented yeasts were recovered by Walker and Ayres (35) from poultry. The genera they isolated included Rhodotorula, Torulopsis, Trichosporon and Candida.

Greater numbers of enterococci than coliforms were recovered from fresh chickens. The presence of Salmonellae on chickens and turkeys undergoing processing was studied. The low numbers generally found were such that their detection was not facilitated by the usual procedure of swabbing only an area 2-10 cm^2. For this reason, larger swabs were used and one entire side of the bird was sampled. The quantity of these organisms/cm^2 recovered from turkeys was generally higher than from chicken. Using selenite F and tetrathionate as the enrichment broth and brilliant green agar and bismuth sulfite as the streaking media, recovery of Salmonellae ranged from 4-15% of the numbers of turkeys sampled.

Sanitation

As may be seen in Fig. 6, the storage life of pieces of chicken was found to depend upon their initial quality. High, moderate, and low count cuts stored at 4° and 10°C varied markedly in keeping quality. While the bacteriological contamination on the live bird contributed to the flora of the eviscerated product, Goresline et al. (14), Drewniak et al. (10) and Walker and Ayres (32) found that sanitation practices adopted in various

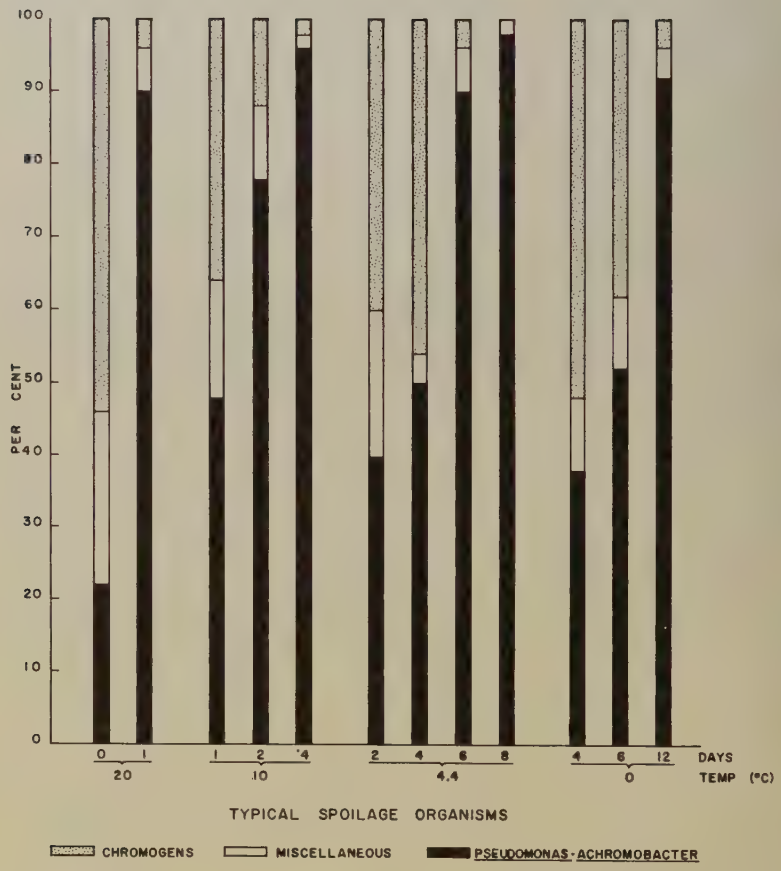

Figure 3. Effect of storage at 0°, 4.4°, 10°, and 20°C on organisms associated with dressed poultry (2).

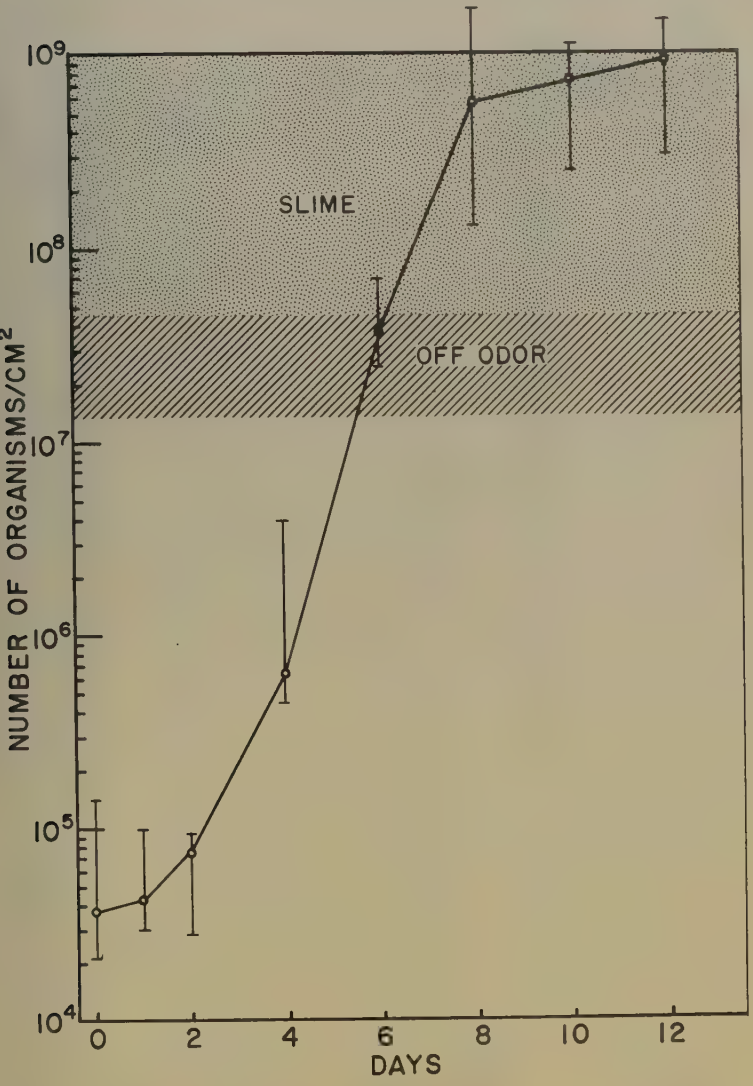

Figure 4. Growth curves showing relation of bacterial numbers, off odor and slime to storage time at 4.4°C.

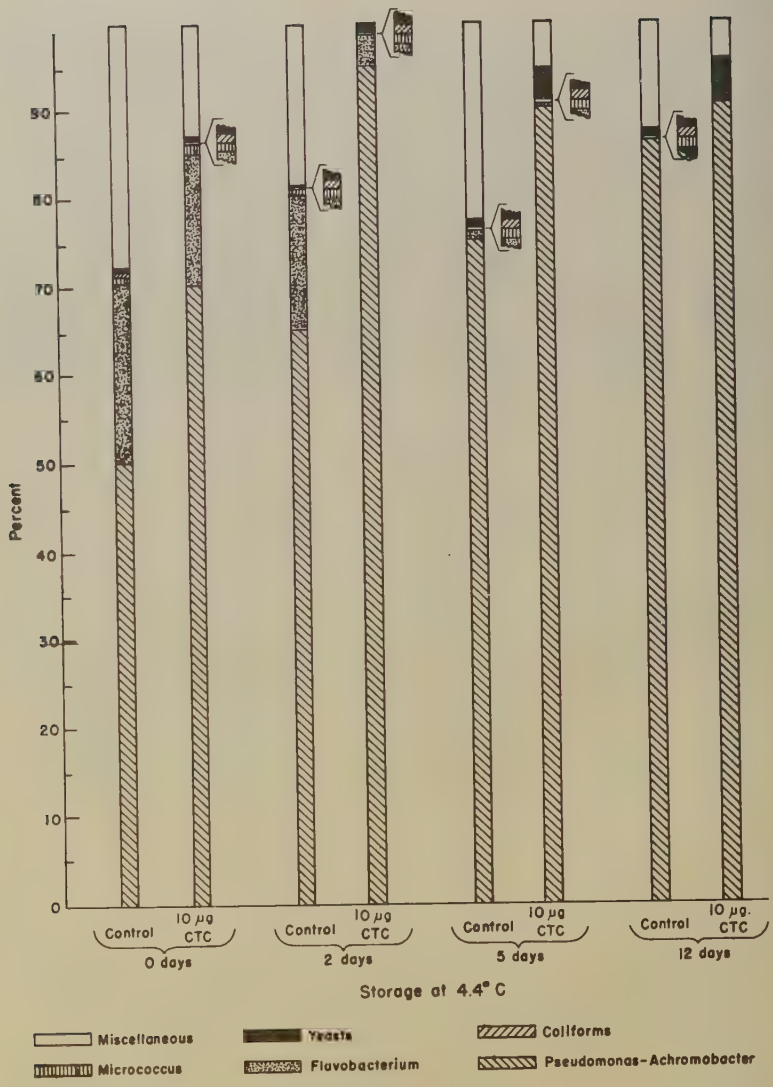

Figure 5. Flora recovered from control and chlortetracycline treated birds after various storage times (3).

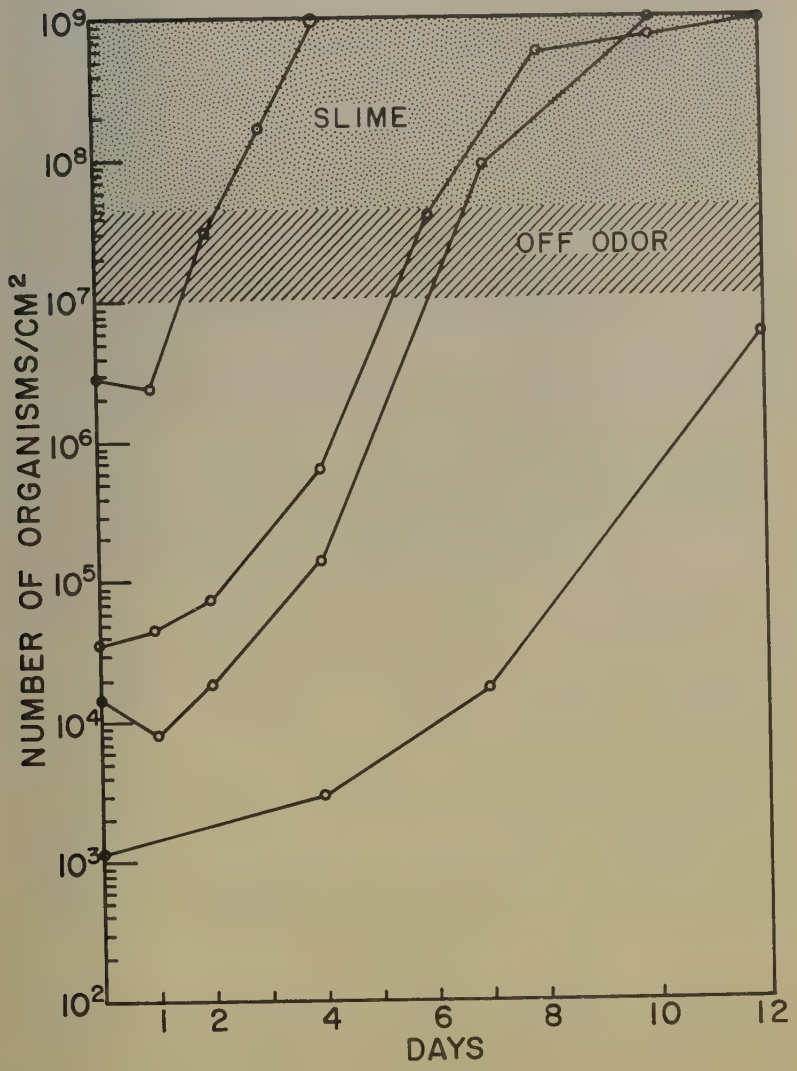

Figure 6. Effect of initial load of microorganisms on time required for development of off odor and slime.

processing plants had marked influence on the numbers of organisms that were recovered. Bacterial loads were lower in some plants than in others. For example, counts from one plant were 4,000 organisms per cm^2 while from another the load was 350,000 per cm^2. Samplings of bacterial populations from the visceral cavity ordinarily revealed loads from 1400 to 12,000 organisms per cm^2. Usually, loads of microorganisms recovered from the visceral cavity were lower than those from the skin. However, one processing line—in which crop removal involved flushing the incision with water and where debris as well as blood were washed into the cavity—gave counts ranging from 54,000 to 93,000 per cm^2. Birds were analyzed at various stages along the processing line. Samples were taken from each of the following: (1) the live bird, (2) the scald water, (3) after the rough picker, (4) after the neck picker, (5) after pinning, (6) after singeing, (7) after evisceration, (8) cavity of the bird, (9) the bird as it was placed in the chill tank, (10) fresh chill tank water, (11) aerated chill tank water, and (12) the final product after chilling. Counts obtained from each of these samplings (Fig. 7) indicated that the numbers of organisms on the skin surfaces tend to increase during processing. Usually, numbers of organisms/cm^2 recovered from turkey were higher than from chicken (36).

The temperature at which fresh poultry is held greatly influences microbial development and shelf life. Ayres, Ogilvy and Stewart (2) found that birds stored at 0°C had a storage life of 16 days; at 5°C birds spoiled at 7 days, and at 10°C off odor and slime were observed at 3 days. Shannon and Stadelman (27) also found that birds stored at 0°C kept considerably longer than those held at higher temperatures. Baker (5) found no difference in bacterial counts or appearance of dry-packed and ice-packed broilers. However, Stadelman et al. (29) reported that holding of chickens in ice for 48 hours prior to cutting into parts resulted in longer shelf life than cutting immediately after cooling. Fromm (13) stated that shelf life of broilers was directly proportional to the time that carcasses were held in slush ice.

Ogilvy and Ayres (26) found that atmospheres containing carbon dioxide improved the storage life. The storage index was a linear function of carbon dioxide concentration. Chicken held in an atmosphere containing 15% carbon dioxide kept twice as long as pieces held in air while those stored in the presence of 25% carbon dioxide kept 2.5 times as long. The maximum proportion of carbon dioxide that could be employed generally was considered to be at a level of 25%. When higher levels were used, discolorations developed. As carbon dioxide concentrations increased, the effect of storage temperature was minimized.

Packaging

Packaging was also found to influence the bacterial population and shelf life of the product. Stewart (30) discussed some of the requirements, advantages, and limitations of several of the more commonly used packaging films. Carlin, Holl and Walker (8) reported lower counts for broilers stored in Cryovac than for those packaged in LSAD-300 cellophane. Eviscerated chicken packaged in polyethylene and stored at 4°C usually developed undesirable odor and slime within 6 days. McVicker and associates (22) found that fryers held in loosely packaged

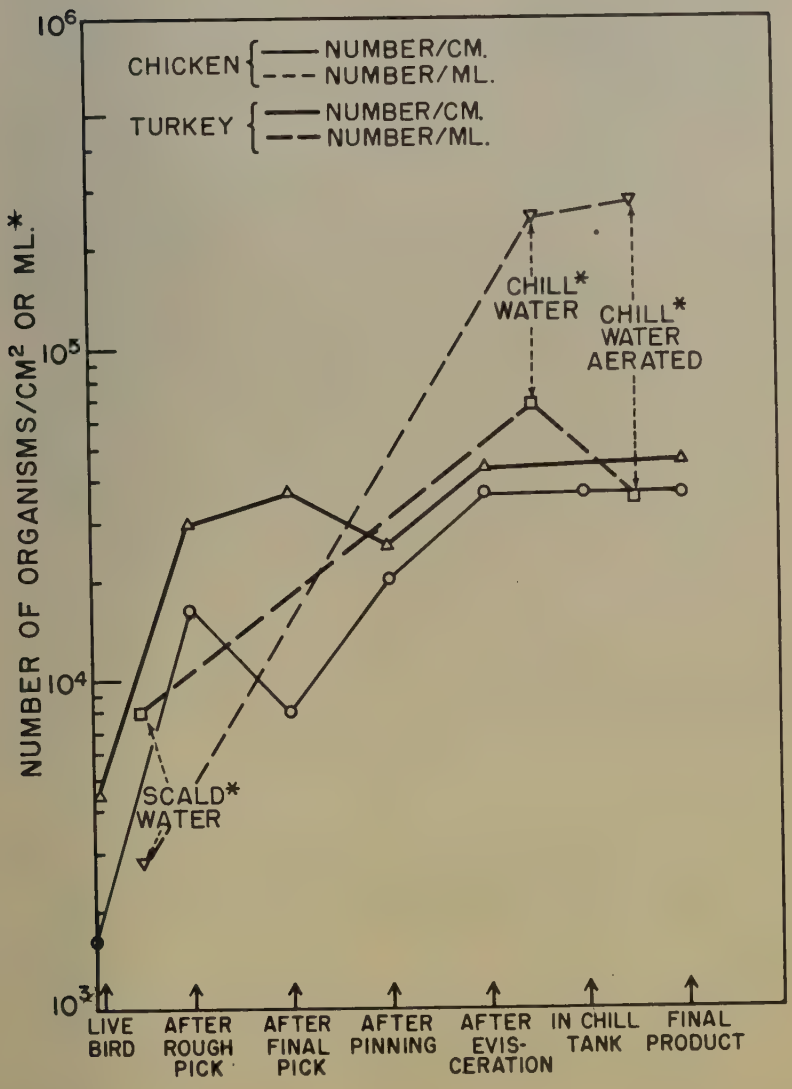

Figure 7. Populations recovered from chickens and turkeys at various stages of processing or from scald and chill tank waters (32, 36).

polyethylene bags had the same shelf life as unpackaged birds but that fryers in tight-fitting packages from which air had been evacuated had longer shelf life than those loosely packaged. Wells et al. (38) did not consider that cellophane or Cryovac packaging films exerted any direct bacteriostatic effect but they stated that the technique of partially evacuating the air from birds wrapped in impermeable films did tend to inhibit bacterial growth by virtue of reduced oxygen tension. Cotterill (9) reported a fluorescence test wherein, upon exposure to ultraviolet, carcasses packaged in polyethylene, pliofilm and cellophane gave a positive reaction while those wrapped in Cryovac did not. There may be some question if this test is used as a sole criterion for determining the superiority of packaging in a tight-fitting film. Since oxygen is required for fluorescence, merely the lack of fluorescence does not indicate that spoilage is prevented but only that the Cryovac process eliminates most of the free air. More work on the evaluation of packaging materials and on tests for determining population changes needs to be undertaken.

Antibiotics

A large number of investigators (1, 3, 5, 6, 11, 19, 22, 23, 27, 28, 29, 31, 37, 40, 42) have studied the practical application of tetracycline antibiotics for delaying microbiological spoilage and thereby lengthening the shelf life of poultry meat. Comparison of flora of control birds and of chlortetracycline treated birds after various times of storage is shown in Fig. 8. Both chlortetracycline and oxytetracycline were found to be equally inhibitory for the bacterial flora (Fig. 9) at a level of 30 μg/ml but at lower levels, e.g. 3 μg CTC delayed growth longer than did OTC or TC. Wells et al. (37) found dips containing 10 ppm chlortetracycline to be more effective in delaying the development of odor and slime than either of the other two tetracycline antibiotics. Data obtained by Vaughn et al. (31) did not differentiate among the three tetracyclines but concluded that tetracycline provided the best over-all performance. Treatment of birds by dipping in water containing any of the tetracycline antibiotics was shown to produce two separate effects:

(1) it reduced the total flora, thereby prolonging the storage life when used once only,

(2) when used continuously in the same equipment over an extended period of time, larger proportions of the populations consisted of pseudomonads and yeasts.

Chlortetracycline (CTC) had little, if any, inhibitory effect on the growth of Pseudomonas or yeasts; at the time that off odor and slime developed, these organisms comprised at least 95 per cent of the population. Yacowitz et al. (40) were among the first to report that there was increased yeast growth and yeasty odor from chicken parts treated with CTC. Ng et al. (24) observed that resistant forms of bacteria prevailed on birds processed in the presence of chlortetracycline. Also, in trials conducted by Walker and Ayres (33, 34) organisms found on poultry treated with chlortetracycline, oxytetracycline or tetracycline were observed not to be as susceptible to these antibiotics as were the bacteria

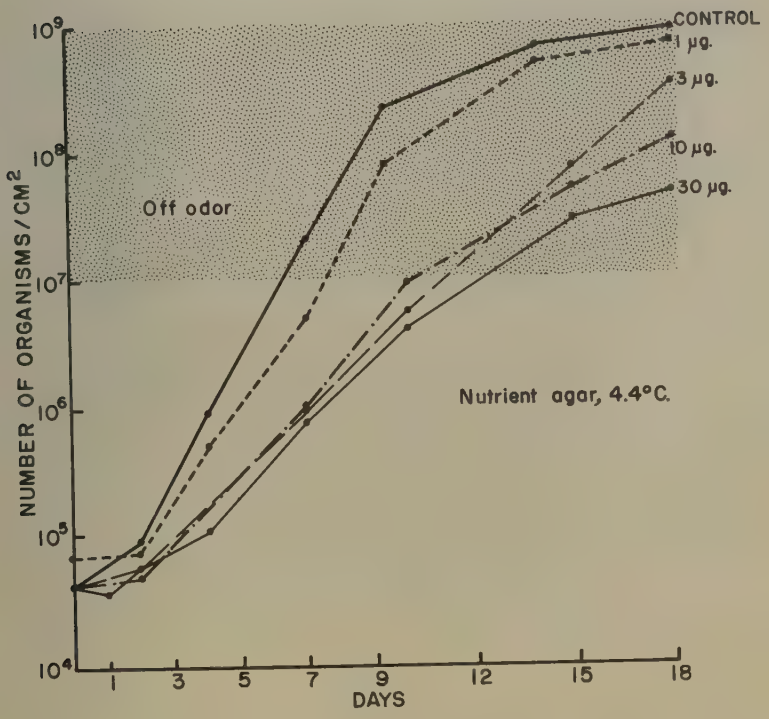

Figure 8. Comparison of numbers of organisms on control birds and on chlortetracycline treated birds after various storage times.

occurring on control birds. Organisms found to be resistant to one of the tetracyclines were also resistant to the other two. Among these, the pseudomonads and yeasts were the most numerous and had the most significance to spoilage later. Growth of Pseudomonas fluorescens and of yeast isolates in mixed culture showed that the maximum number of yeasts was not as high as when the yeasts were growing in the absence of the bacteria. When CTC was added to a mixture of the organisms, the number of yeasts was equal to that found in the control (yeast only). The presence of yeast had no apparent effect upon the growth of the bacteria. Addition of CTC to a mixture of yeasts and pseudomonads or pseudomonads alone caused a lag in the growth of the bacteria. It would appear that the increased numbers of yeasts on antibiotic treated poultry can be explained primarily on the basis of decreased numbers of bacteria which otherwise suppress the growth of yeasts.

Recognizing the limitations that use of antibiotic treatment had against pseudomonads and yeasts when chlortetracycline was used alone, Ayres

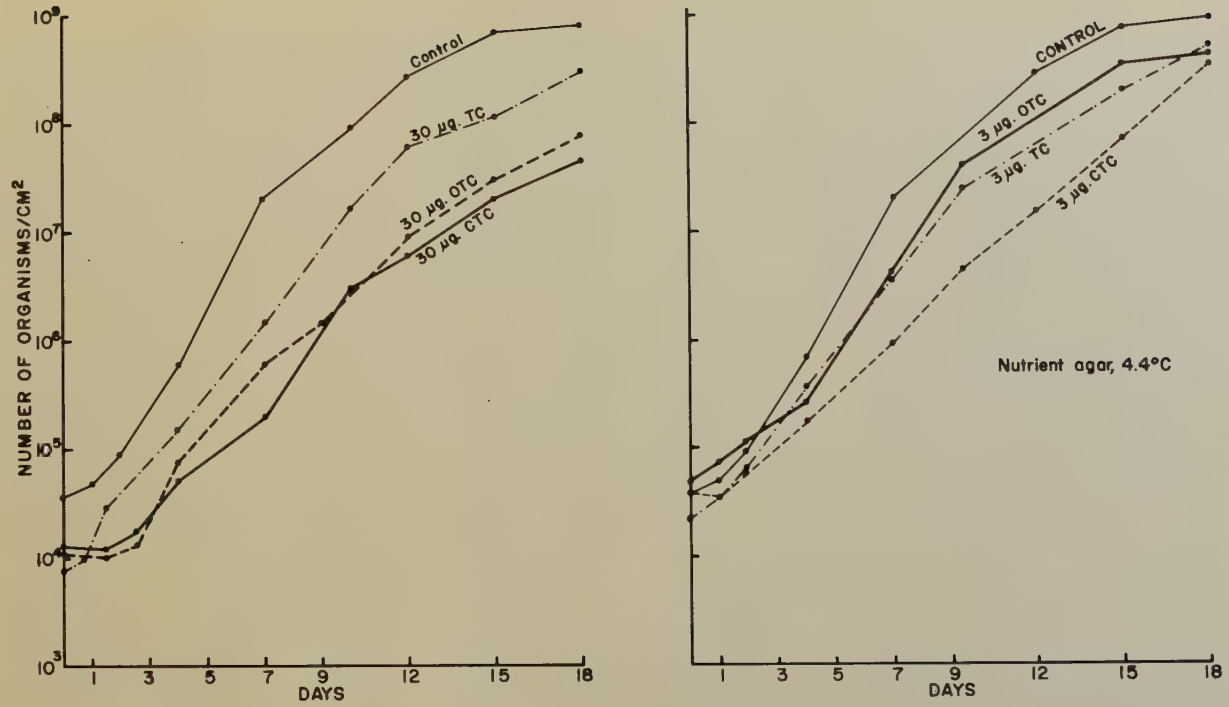

Figure 9. Effect of tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) on numbers of bacteria.

et al. (3) used combinations of chlortetracycline with various other agents such as streptomycin, neomycin, nystatin, aerosporin, myprozine, ascosin and rimocidin. When chlortetracycline was used with neomycin or nystatin (Fig. 10), it was effective in reducing the populations of pseudomonads. When used in combination with myprozine (Fig. 11), the complementary action of the two agents retarded the development of both bacteria and fungi. Tetracycline residuals recovered from the skin of antibiotic treated chicken meat were found to decrease rapidly during the first 48 hours. After that time, however, the concentrations were much more constant and detectable amounts could be found for at least 21 days. Losses in the deep tissue were much less pronounced and, in all trials, persisted at higher levels than on the surface. There appeared to be no preferential absorption of the antibiotics. The levels that persisted depended upon the concentration of antibiotic used for treatment and upon the length of time of exposure to the antibiotic.

It is probably safe to assume that the amount of chlortetracycline residue permitted in raw chicken (7 ppm) is sufficiently low that the chemical is not active after cooking. However, with red meats receiving much less drastic cookery, i.e., frankfurters and ground meat patties, Escanilla, et al. (12) found that this was not true. Ground meat patties cooked to a final internal temperature of 71°C (well-done stage) still had residual chlortetracycline and that the amount of active tetracycline remaining was a direct function of the initial concentration in the raw ground meat. They concluded that, regardless of the treatment level of CTC used, the antibiotic was never completely inactivated.

Recently, Meyer et al. (23) reported that agar and carrageenin gels increased the keeping time of eviscerated poultry when the tetracycline antibiotics were incorporated in the coatings and also that combinations of these with other antibiotics were more effective than were the tetracyclines alone. Their studies provide a possible vehicle whereby antibiotics may be applied without exposing the bird to subsequent contamination. A possibility being tried in this laboratory is the incorporation of the tetracycline antibiotics in the formulation of packaging films; this study is still in progress.

SUMMARY

A comparison of rinse, cut-section and swab sampling methods for estimating numbers of organisms on poultry skin indicated that results by these three procedures, while not identical, were comparable and that use of moist swabs was the easiest, most rapid and economical of the tests. Also, the modified resazurin reduction test was found useful in determining the quality of pan-ready chicken.

Microorganisms belonging to several genera were recovered from the skin of freshly killed birds. However, a uniform psychrophilic flora consisting predominantly of pseudomonads developed a few days after processing and caused off odor and slime.

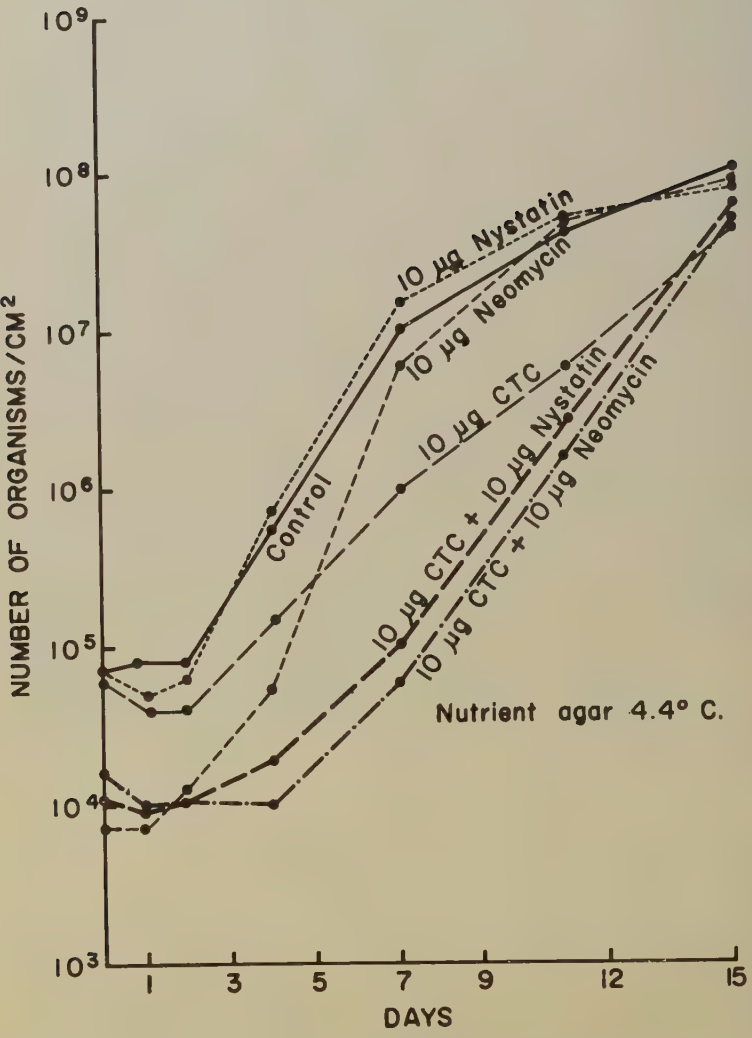

Figure 10. Effect of chlortetracycline alone or in combination with neomycin or nystatin on bacterial populations.

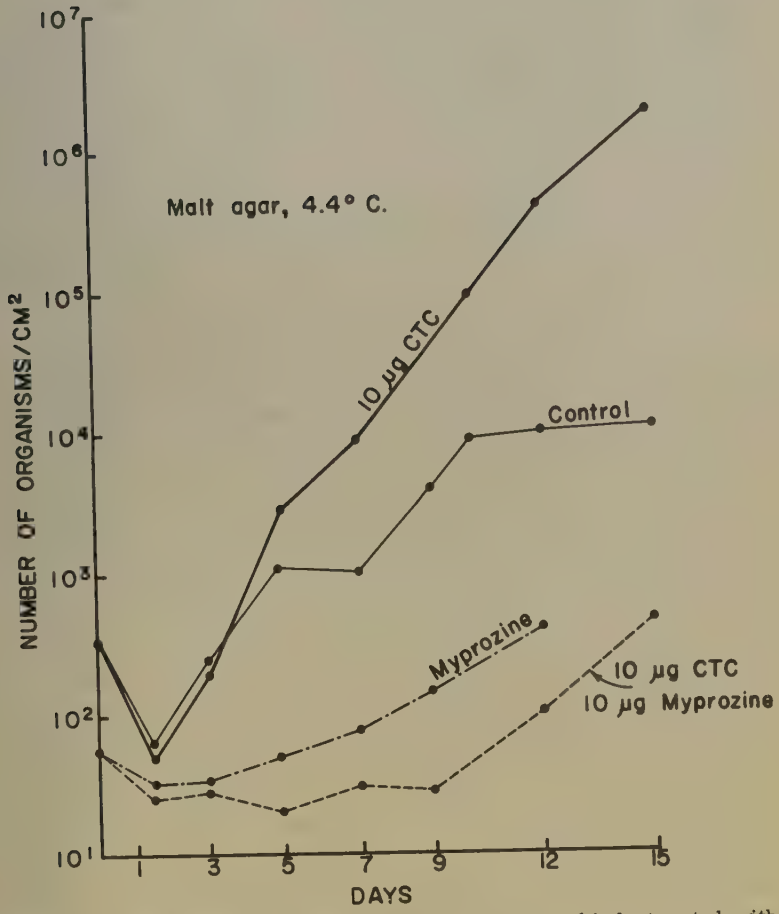

Figure 11. Comparison of yeast counts obtained on birds treated with chlortetracycline and myprozine.

While the storage life of chicken was found to depend upon its initial quality, the sanitation practices adopted in various processing plants markedly influence the microbial populations. Usually fewer microorganisms were recovered from the visceral cavity than from the skin. Numbers of organisms on skin surfaces tended to increase during processing.

Holding temperatures greatly influenced microbial development and storage life as did also packaging and use of atmospheres containing carbon dioxide.

The tetracycline antibiotics reduced the total flora but when used on a continuous basis had very limited value. While pseudomonads and yeasts were found to be more resistant than other organisms to these antibiotics there was no direct evidence that the tetracyclines stimulated growth. Less growth of yeasts was observed in mixtures containing pseudomonads; the presence of yeasts, however, had no apparent effect upon growth of the bacteria.

When one of the tetracyclines was used in combination with nystatin or neomycin, concomitant action of the two antibiotics reduced populations of pseudomonads. When chlortetracycline was used with myprozine, complementary action of the two agents retarded the development of both bacteria and fungi. Incorporation of these antibiotics in agar or in Pliofilm may have value in prolonging storage life of chicken.

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CULTURAL FACTORS IN SEEDLING VIGOR OF SMOOTH BROMEGRASS AND OTHER FORAGE SPECIES[1]

R.R. Kalton, R.A. DeLong, and D.S. McLeod[2]

Successful stand establishment is of paramount importance to profitable use of forage crops in any farming system. Despite considerable research over a period of years, establishment problems still are common in many forage species. Several factors are known to influence the ability of any seeding to establish a good stand. Some of these are: time and method of seeding, soil moisture and fertility conditions, type of companion crop, temperature, depth of seeding, seed quality and viability, seed size, species and variety used, soil type and structure, and fertilizer treatments. Most research to date has been concerned with species, equipment, climatic, soil and date of planting effects. Moreover, many studies have been conducted under greenhouse or laboratory conditions without relating results to field responses. Relatively few intensive investigations have been made concurrently of genotypic, seed size, soil conditioner and planting depth factors and their possible interrelationships under both greenhouse and field conditions. The present study was concerned with these latter factors and their effects on early stand establishment in smooth bromegrass and four other forage species of major importance in Iowa.

Investigations involved six different experiments relating to seedling vigor in its broadest sense. Three experiments included comparisons of species at three planting depths in the greenhouse and field following the use of Krilium or PR-51 as soil conditioner treatments. Species used were smooth bromegrass, timothy, alfalfa, red clover, and birdsfoot trefoil. The fourth experiment consisted of five strains of bromegrass planted at three depths with and without soil conditioner treatment. The last two experiments were concerned with strains of bromegrass differing widely in seed size and planted at different depths in the field and greenhouse. Seedling vigor responses were determined by evaluation of rate and amount of emergence, and vigor of early seedling growth.

Major aims were to determine possible differential responses of species and bromegrass strains to planting depth and soil conditioner treatments and effect of seed size in brome grass on seedling vigor attributes. Relationships of greenhouse and field responses also were of considerable interest in regard to development of future studies of stand establishment factors. For bromegrass in particular, it was hoped to

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[2] Professor of Farm Crops and former graduate students, Department of Agronomy, Iowa State College, respectively.

obtain information that could be applied to a program of breeding for improved seedling vigor and seed size. Additional research results regarding this phase will be presented in a second paper on genetic factors in seedling vigor of bromegrass.

REVIEW OF LITERATURE

Willard (36) discussed a number of limiting factors in establishment of forage seedings. Considered important in germination of live seed were sufficient air and moisture, favorable temperature and permeable seed coat, while drying, freezing, insufficient or too deep coverage, and crusted soil surface commonly limited establishment (or emergence) of seedlings. Failure of seedlings to grow after establishment could be due to lack of inoculation, inadequate lime or plant nutrients, poor drainage, drought, competition from companion crops or weeds and insect or disease damage. Suggestions based on research results were made for overcoming these limitations. Other important factors observed to affect establishment are species differences (7, 9, 28, 29), seed size (22, 33), age of seed (13, 17), light intensity (12, 14), and strain differences within species (4, 14, 15, 16, 21, 30, 31, 35). In the present review only literature pertinent to stand establishment factors studied herein will be considered in any detail.

Effect of planting depth on seedling vigor in various forage species has long been a subject of investigation. In 1932 Love and Hanson (25) noted that both crested wheatgrass and bromegrass could emerge from depths up to 3 inches, but best emergence occurred at $\frac{1}{4}$ to $\frac{1}{2}$ inch for the former and from $\frac{1}{4}$ to 1 inch for the latter. Murphy and Arny (29) compared rate and percentage of emergence of a number of forage species at surface, $\frac{1}{2}$, 1, 2, and 3-inch planting depths on five soil types. Generally, legumes emerged faster than grasses in both greenhouse and field, indicating a need for a longer period of favorable conditions for establishment of grasses. Depth of planting appeared to be the most important factor in total emergence with the $\frac{1}{2}$-inch depth satisfactory for most species. Timothy, alsike clover and white clover emerged best at $\frac{1}{2}$ inch and mostly poor at 1 inch, while alfalfa and sweet clover sometimes showed good emergence at 1 inch. Red clover and reed canary grass gave good stands on all soil types at the 1-inch depth, while bromegrass showed satisfactory emergence when seeded up to 2 inches deep on all but one soil type. In a similar study Moore (28) also found a differential emergence response of forage species to planting depth on various soil types. He obtained optimum germination and emergence with $\frac{1}{4}$ and $\frac{1}{2}$-inch depths for most species, though larger-seeded species emerged satisfactorily from somewhat deeper plantings on lighter soils.

Plummer (32) compared emergence of 12 range grasses, including bromegrass, at surface, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1 and $1\frac{1}{2}$-inch seeding depths. Surface planting, however, gave poor germination for most species. Best depths were $\frac{1}{4}$ and $\frac{1}{2}$ inches if sustained favorable moisture conditions prevailed. Several other investigators (1, 2, 3, 11, 17, 24, 33, 35) have studied effects of planting depth on rate and percentage of emergence and on early seedling growth in forage crops. Generally, best results were obtained with

shallow ($\frac{1}{4}$ to $\frac{1}{2}$ inch) seedings, though differential responses to depth were found among strains within species (1, 24, 33, 35).

Seed size, weight and quality are other factors known to influence seedling vigor. Seed weight is of particular interest in the present study. Almost 40 years ago Kidd and West (20) pointed out that parental differences and environmental factors affecting them can influence seed size and that larger seeds may give rise to more vigorous plants and a better yield. They also extensively reviewed early literature pertaining to physiological predetermination of seed condition and its effect on subsequent growth and yield. Hermann and Hermann (17) found that seed weights, emergence from different planting depths and heights of seedlings all increased as seed was harvested closer and closer to maturity. They noted that viable seeds could be obtained by harvesting earlier than the hard dough stage in crested wheatgrass but such seed did not produce vigorous seedlings. McAllister (27) harvested seeds of several range and pasture grasses at several stages from pre-milk to maturity and tested germination over a 58-month storage period. Immature seed did not emerge as well under field conditions as mature seed and did not retain germination as well in storage. Grabe (13) conducted an intensive study on effect of stage of maturity at seed harvest in bromegrass on seed size, viability, and seedling vigor. Maximum dry weight of seeds and full germination potential were reached by 17 to 18 days after anthesis. It was found that ability to germinate and seedling vigor were directly proportional to seed weight and, therefore, to amount of food reserves contained in the seed.

Relationships of seed weight or size to seedling vigor have received attention in a number of instances. In 1927, Davies (9) reported correlations between 1000 seed weight and percentage stand establishment ranging from 0.698 to 0.795 for a number of forage species and tests. Plummer (32) noted that heavier-seeded grasses emerged faster and better from deeper depths of planting than light-seeded species. Murphy and Arny (29) likewise found strong correlations between seed weight and total emergence from deeper planting depths for 18 forage species. With alfalfa, Erickson (11) found a direct association of germination and seedling vigor with seed size, though effect on seedling vigor decreased with age of seedlings. Small seeds resulted in more decreases in vigor than large seeds with increased depth of seeding. Beveridge (1) compared small, medium and large seeded lots of alfalfa seed at three depths of seeding and found the most vigorous seedlings at any depth came from large seeded lots. Large seeded types tended to emerge faster, though no significant correlation of seed size with rate or amount of emergence was noted. Black (3, 4, 5) conducted some very detailed studies on seed size with subterranean clover. He noted that seed size determined depth from which emergence took place and initial cotyledonary area. However, for any one seed size, plants grew at the same relative rate after emergence irrespective of planting depth. In early stages of seedling growth both dry weight at any sampling date and leaf area were linearly related to seed weight. These differences in early seedling weight in favor of large seeded lots were maintained in spaced plantings up to 194 days after emergence. With close plant spacings, differences persisted only up to about 88 days after emergence when competitive effects slowed

growth. At 155 days plants from all seed size classes were equal in dry weight.

Working with a number of grasses in Scotland, Hunt (18) pointed out that seed weight could be affected by strain, relative maturity, harvesting conditions and degree of cleaning. Wide differences in seed size among strains of ryegrass, meadow fescue, timothy and orchardgrass were found and heavier seeded types tended to be higher in percentage of stand establishment. Rogler (33) studied relationship of seed size to emergence from different planting depths in crested wheatgrass seed lots. At 2 inches or deeper, a strong positive correlation between size and emergence was noted. Larger seeded types also gave more vigorous seedlings at the deeper depths. Kneebone (21) and Kneebone and Cremer (22) related seed size to seedling vigor traits in several range grasses and generally found large-seeded samples best in rate and amount of emergence and in seedling size. Breeding for larger seed size to improve seedling vigor was suggested in all of the latter three papers. Somewhat contradictory results on seed size were obtained by Lawrence (24) with 24 lines of intermediate wheatgrass. Though lines differed in seed weight and emergence from different planting depths down to 3 inches, emergence generally was unrelated to seed size.

Peace (31) measured seed weights of 21 bromegrass strains from a variety test in Montana and checked samples for seedling vigor. Genotypic correlations between seed weight and seedling vigor were 0.775 one year and 0.745 the next. Tossell (35) also found that bromegrass seed lots with high seed weight tended to be high in factors of establishment. In addition, seed weight classes within a seed lot differed in percentage stand and vigor. A correlation of 0.90 between seed weight and seedling vigor of 6 brome varieties was obtained by Hawk and Welch (16).

Wide differences in seedling vigor attributes among strains, seed lots and even individual selections, aside from seed size effects, have been observed (14), though the significance of genetic factors only recently has been appreciated. Results of this nature were obtained with alfalfa by Beveridge (1), with several range grasses by Kneebone and Cremer (22), with intermediate wheatgrass by Lawrence (24) and with bromegrass by Hawk (15) and Peace (31). Newell and Keim (30) noted that northern strains of bromegrass, though excellent in seed quality, often produced weak seedling growth in fall seedings in the field, while southern seed sources from Nebraska and Kansas were very good in seedling vigor. Hawk and Welch (16) noted the same response of the two types and conducted a greenhouse study with several strains of each ecotype with and without seed treatment in pythium-infested and noninfested soil. Northern strains were somewhat better in seedling vigor in sterilized, noninfested soil than southern varieties but the reverse was true in infested soil. Varietal differences in seedling vigor within each group also were observed. A number of strains, polycross progenies and open-pollination progenies of bromegrass were found to vary widely in rate of emergence, maximum stand, height and vigor by Tossell (35) and not all differences could be ascribed to seed weight. A somewhat different observation was made in subterranean clover by Black (4) who found that, though three strains differed in seedling weight at any one time due to initial seed weight differences, relative growth rate was identical.

Another aspect of seedling vigor is rate of growth after establishment which Willard (36) indicated could be affected by several factors including species differences. Love and Hanson (25) noted that bromegrass grew faster than crested wheatgrass the first 24 days after planting but crested wheatgrass was superior thereafter. Plummer (32) found wide differences among species in root and shoot development in early stages growth. Bromegrass was one of the best in root growth the first 28 days in the greenhouse, while crested wheatgrass was outstanding in root development in the field the first few months after seeding. A close relationship between rate of root development in the seedling stage and subsequent establishment was indicated. Competition with other species and weeds can have a marked effect on amount of seedling growth, as found in studies by Blaser, et al. (6, 7). In the field relative aggressiveness of seedling growth was rated at 23 to 42 for orchardgrass, 43 to 52 for bromegrass, 17 to 18 for timothy, and 28 for reed canarygrass compared with 100 for alfalfa. Seedling weights varied with sampling dates with the legumes similar in relative growth at 36 and 51 days after seeding. Relative growth rate of orchardgrass, however, increased markedly between 36 and 51 days, while that of bromegrass increased slightly. Relative growth of bromegrass in spring and fall seedings in comparison with alfalfa was similar, while orchardgrass was twice as good in the spring as in the fall. Differences among bromegrass varieties in seedling aggressiveness were observed in one test. In mixtures (6), aggressiveness of seedlings of species involved varied, depending on season of planting. Red clover seedlings developed faster in spring than in late summer plantings, while alfalfa was the reverse. Orchardgrass was about equal for the two seasons. Percentage stand establishment for orchardgrass and alfalfa was similar for the two dates but much better for red clover in the spring. Both red clover and orchardgrass seedlings were more competitive with alfalfa in spring than late summer seedings. Consequently, botanical composition in subsequent harvest years varied widely for the two seasons of planting due to changing competitive abilities, even though total average yields were the same.

Krenzin (23) studied the relationships between variety, seeding rate and management of the oat companion crop on forage crop stands and yields. The oat varieties, though different in maturity, did not differ in effect on stand or yield. Competition from weed growth in the absence of a companion crop was more severe than from oats. Legume stands were higher and forage yields increased when oats were removed at hay stage rather than at maturity. In the greenhouse Krenzin (23) compared growth responses of alfalfa, birdsfoot trefoil and orchardgrass grown alone and in grass-legume combinations at 30, 60, 90 and 120 days after seeding. Alfalfa grew faster than trefoil from 30 to 90 days after seeding, while orchardgrass exceeded both legumes during the same period. Species were similar in growth from 0 to 30 days and from 90 to 120 days. In the associations, orchardgrass suppressed legume yields, with greater effects on trefoil than alfalfa. However, greater yields of orchardgrass more than offset reduced yields of the legume. No evidence of either antagonistic or mutually beneficial effects from associations was obtained.

Alteration of seedling vigor and competitive ability of forage species

by light intensity, soil and air temperature, nutrient and moisture availability, day length, diseases, soil type and other environmental factors may occur (2, 7, 8, 12, 15, 16, 29, 35). Chippendale (8) noted that timothy germinated poorly at low temperatures while orchardgrass did well. At higher temperatures timothy germinated quickly but seedlings were small and grew slowly. In a study of pre-emergence weight changes in subterranean clover, Black (2) noted that hypocotol extension per unit of cotyledonary reserves increased from 7° to 14° to 21°C., which was optimum. Gist and Mott (12) found red clover seedlings produced more top growth than alfalfa under low light intensities. At higher light intensities, alfalfa top growth exceeded red clover the first 10 to 40 days after emergence but red clover was better from 41 to 56 days. Root growth of alfalfa was low at low intensities but better than that of red clover at high light intensities at all stages. Generally, growth responses of alfalfa, red clover, and birdsfoot trefoil were linear for varying light intensities, though trefoil was only about one-third as good as alfalfa in seedling growth. Hawk (15) found that several strains of bromegrass produced more seedling growth in a warm greenhouse under a long day (18 hours) than under a normal day length inside or outside the greenhouse in flats in a fall seeding. No differential response among strains to day length or temperature treatments was noted, nor did treatments affect stand percentage.

Keller (19) compared growth responses of three timothy strains, varying in maturity from early to very late, and an early and a late red clover strain in pure stands and in associations under 10, 14 and 18 hour photoperiods in the greenhouse. Generally, yields of all variety combinations increased with longer photoperiods. Association with red clover tended to favor growth of the timothy varieties under 14- and 18-hour days, indicating a greater competitive ability for timothy under longer photoperiods. The early timothy variety was most competitive under a 14-hour day length. Vegetative growth of red clover was less sensitive to varying photoperiods than timothy and the early red clover responded better in associations at the 14-hour day length. The late red clover tended to be superior at the longest photoperiod. Results generally indicated that varieties may respond differentially in seedling growth to varying day lengths.

Over 30 years ago, Davies (9) noted that heavier soils tend to give poorer stands of forage species than lighter soils and that fertilizer improved seedling vigor in some species. Since then, numerous studies have shown that fertilizers may improve seedling vigor under many circumstances. As an example, Blaser et al. (7) recently noted that nitrogen fertilizer increased aggressiveness of orchardgrass seedlings relative to alfalfa in spring seedings. Development of synthetic soil conditioners, which may improve soil structure and workability, reduce crusting, lessen erosion hazards, and increase permeability (26, 34), has brought another factor into possible consideration for improving forage stand establishment. Though use of such treatments has improved emergence for certain field and horticultural crops in some tests (10), Sherwood and Engibous (34) pointed out the need for additional research as to effect on seedling emergence. Reports concerning effects of soil conditioners on seedling vigor traits in forage species apparently are rare in present-day literature.

A final point of interest relating to seedling vigor research in forage species is the degree of relationship between greenhouse and field results. Kneebone and Cremer (22) found that differences among species and among seed size lots within species were not as pronounced in the field as in the greenhouse. Davies (9), Rogler (33) and others have noted that field germination and rate of emergence often are slower than in the greenhouse. However, the latter obtained a correlation of 0.90 between field and greenhouse emergence ratings for a number of crested wheatgrass seed lots planted at six depths. Beveridge (1) obtained fastest emergence at the $\frac{1}{2}$-inch depth of seeding of a number of alfalfa seed lots in the greenhouse but in the field, the 1 and $1\frac{1}{2}$-inch depths emerged about as fast as the $\frac{1}{2}$-inch depth. Good agreement between greenhouse and field trials for seed quality and seedling vigor in a number of bromegrass lines was obtained by Tossell (35). Despite these results, it is possible that greenhouse data may often be unreliable for predicting field performance.

MATERIALS AND METHODS

The present investigations consisted of six different experiments to evaluate effects of several cultural factors on seedling vigor. Three experiments were conducted in the greenhouse and three in the field, with one soil type, Webster silty clay loam, being used throughout. Cultural variables involved were species, strains and varieties of bromegrass, planting depths, soil conditioners, seed weight in bromegrass, and time of planting. However, not all were considered in each experiment. For clarity, the components of each experiment are summarized in Table 1.

Seed for Experiments I, II, and III was high in quality and obtained from the Iowa State Seed Laboratory. The same lot of seed of each species was used for all three tests. Bromegrass seed for Experiments IV, V, and VI was produced at Ames in most instances except for seed of a few varieties obtained from respective states of origin. All greenhouse plantings were made in 21 x 14 x 3-inch wood flats using sterilized soil. Flats were placed on inverted pots on wood benches to reduce dangers of seedling blight infection and rotated daily within replications to minimize environmental effects. Soil was kept moist by regular watering. All field plantings were made on well-prepared soil using a Planet, Jr. garden seeder. Planting depths in the field were obtained by adjustment of the planter shoe. After sowing, all plots were firmed with a double roller. In both greenhouse and field Krilium (Formula 6) was applied in an equivalent rate of 800 lbs per acre on a dry basis and thoroughly mixed with the first few inches of surface soil. PR-51 was applied at the rate of 30 lbs per acre by dissolving the equivalent amount in water and applying it to the soil with a sprinkling can.

In Experiments I and II in the greenhouse a split-split plot design with planting depths as whole plots, soil treatments as subplots and species as sub-subplots was used. Each whole plot was replicated four times and consisted of one flat. Each flat was divided with a wood or metal divider strip into subplots for soil conditioner treatments. Sub-subplots

Table 1. General summary of experiments conducted to measure effects of several factors on seedling vigor.

Experiment No.	Entry descriptions	Location	Planting depth (inches)	Soil conditioner treatments	Date planted
I	Bromegrass, timothy, alfalfa, red clover, birdsfoot trefoil	Greenhouse	$\frac{1}{2}$, 1, $1\frac{1}{2}$	None, Krilium	1/27/53
II	Bromegrass, timothy, alfalfa, birdsfoot trefoil	Greenhouse	$\frac{1}{2}$, 1, $1\frac{1}{2}$	None, Krilium, PR-51	3/17/53
III	Same as Experiment I	Field	$\frac{1}{2}$, 1, $1\frac{1}{2}$	None, Krilium, PR-51	4/21/53
IV	5 varieties and strains of bromegrass	Field	$\frac{1}{2}$, 1, $1\frac{1}{2}$	None, Krilium	4/21/53
V	4 varieties and 8 topcrosses of bromegrass varying widely in seed weight	Field	$\frac{1}{2}$, 1, $1\frac{1}{2}$, 2	None	9/23/55
VI	Same as Experiment V	Greenhouse	$\frac{1}{2}$, 1, $1\frac{1}{2}$, 2	None	11/23/55

of individual species were each planted with 100 seeds in single rows approximately 10 inches long and $1\frac{1}{2}$ inches apart. Seedling vigor evaluations were based on number of days for emergence, which were noted daily, and percentage of emergence read 22 to 24 days after planting.

A similar split-split plot design was used for Experiments III and IV in the field. Individual sub-subplots consisted of a single, drilled row of a species or variety, 8 feet long, with a 1-foot row spacing. Subplots, which consisted of soil conditioner treatments, were separated from each other by two border rows of alfalfa. The planter was calibrated to seed approximately 5 lbs per acre of birdsfoot trefoil and timothy, 10 lbs per acre of alfalfa and red clover, and 15 lbs per acre of bromegrass. Thus, number of seeds planted per plot was not uniform and varied with the species. Observations were made daily to record number of days for emergence, while stand counts were taken on the basis of number of seedlings in the center three feet of each row at 32 to 36 days after planting.

In both field experiments weeds were allowed to grow to provide competition similar to a companion crop. Then, on July 6, 1953 all plots at the $\frac{1}{2}$-inch depth of seeding were carefully weeded and all above-ground parts of each plot clipped for yield determinations. Seedling forage from each plot was dried and yields recorded in grams per plot.

The plant material studied in Experiments V and VI consisted of three varieties (Fischer, Canadian, and Southland), one Iowa synthetic strain (BR-3) and topcross progenies of eight selected clones. Seed of these strains previously had been measured for differences in seed weight per 300 seeds and found to differ widely. Both experiments were planted in a split-plot arrangement with the four depths of planting as whole plots and the 12 entries as subplots. Each whole plot (depth) was replicated four times. Field plantings were made as single-row plots, 6 feet long and 2 feet apart, for each seed entry. Planting rate was calibrated to approximate 15 lbs per acre. Data were taken on number of days for emergence, number of seedlings per 4 linear feet of row in the center of each plot and on seedling weight 60 days after planting. To eliminate stand differences, 25 randomly selected plants were cut off at ground level in each row, dried and weighed.

In Experiment VI in the greenhouse, the 12 seed entries for any one depth of seeding were sown in single rows in one flat at a rate of 50 plump seeds per plot. Data were taken on days for emergence, stand count, and seedling weight 49 days after planting. To eliminate stand differences, 20 plants from each row were cut off at ground level, dried and weighed.

All data from each experiment were subjected to analyses of variance to determine statistical significance of mean differences and factor interactions. Degree of association between field and greenhouse tests and between seed weight and seedling vigor criteria was evaluated by correlation coefficients. Seedling weights and stands obtained in Experiments III and IV were analyzed by covariance analyses and yields adjusted for stand differences where appropriate. In all tests, date of emergence was recorded as the day when an appreciable number of seedlings had emerged.

EXPERIMENTAL RESULTS

In presentation of results, data for each experiment are first given separately to avoid confusion. Interrelationships between greenhouse and field performance and between seed size and seedling vigor in bromegrass are presented in subsequent sections. General implications of findings in regard to stand establishment and future research in this area are presented in the discussion. No analyses of variance are reported for the various experiments, but statistical significance, where found, is indicated by appropriate levels of probability.

Experiment I

In this experiment five species were sown in January at three depths in untreated and Krilium-treated soil in the greenhouse. Means for days to emergence and percentage of emergence for each variable studied are presented in Table 2. As an average, increasing planting depth from $\frac{1}{2}$ to 1 inch and from $\frac{1}{2}$ to $1\frac{1}{2}$ inches delayed emergence by 1.8 and 3.4 days, respectively. Timothy was slowest and alfalfa fastest in emergence at all depths with bromegrass, red clover and trefoil intermediate in this regard. Both mean depth effect and species differences were significant (1% level). No significant or consistent effect of Krilium on rate of emergence was noted for any depth or species and species responded similarly to increasing depths.

Effects on stand percentage were more pronounced. Average emergence declined from 78.7% at $\frac{1}{2}$ inch to 67.4% and 41.0% at the 1 and $1\frac{1}{2}$-inch depths, respectively. Alfalfa was highest, while timothy and trefoil were lowest in average stand percentage. However, part of this difference was due to a differing seed viability noted at the $\frac{1}{2}$-inch depth and persisting at greater depths. Mean differences among depths and species were significant (1% level). Krilium significantly (5% level) improved emergence, on the average, with a 66.9% mean stand compared with 57.8% for no treatment over all depths and species. Improvement of stand due to Krilium, however, was apparent only at the 1 and $1\frac{1}{2}$-inch depths. The difference in stand percentage at the 1-inch depth compared with the $\frac{1}{2}$-inch depth, was only 6% in Krilium-treated soil and 22% in untreated soil. At $1\frac{1}{2}$ inches, decreases from the $\frac{1}{2}$-inch depth were 35 and 61% for Krilium and check treatments, respectively. A significant (1% level) differential response in emergence of species to planting depths was noted with a much greater effect of deeper planting depths on stands of timothy and trefoil than on bromegrass or alfalfa.

Experiment II

In the second experiment PR-51 was added as a soil treatment and red clover was not included; otherwise, this test was the same as the first. Plantings were made in March in the greenhouse and readings taken on days to emergence and stand percentage. Data for these traits appear

Table 2. Mean number of days to emergence and percentage of emergence for five forage species planted at three depths in untreated and Krilium-treated soil in the greenhouse.

Species	½-inch depth		1-inch depth		1½-inch depth		Species mean
	Check	Krilium	Check	Krilium	Check	Krilium	
	Days to emergence						
Bromegrass	6.0	6.2	7.5	7.2	9.8	8.2	7.5
Timothy	7.5	7.5	10.5	9.5	12.5	11.8	9.9
Alfalfa	4.0	4.2	5.5	6.0	7.2	6.5	5.6
Red clover	5.2	5.2	6.8	6.8	9.2	8.5	7.0
Trefoil	5.5	6.0	7.8	7.2	7.8	9.2	7.2
Treatment mean	5.6	5.8	7.6	7.4	9.3	8.8	
Depth mean	5.7		7.5		9.1		
	Percentage of emergence*						
Bromegrass	74.2	69.8	68.0	68.8	46.5	68.0	65.9
Timothy	84.0	75.2	38.2	60.5	5.0	12.2	45.9
Alfalfa	90.2	91.8	81.5	86.8	53.8	82.0	81.0
Red clover	83.8	84.5	79.8	84.0	37.8	64.8	72.4
Trefoil	66.5	66.8	43.8	63.0	13.8	26.0	46.6
Treatment mean	79.8	77.6	62.2	72.6	31.4	50.6	
Depth mean	78.7		67.4		41.0		

*Final reading 24 days after planting.

in Table 3 and show effects of planting depths on emergence generally similar to those in Experiment I. Rate of emergence was somewhat faster, probably due to higher greenhouse temperatures. Also, delays in emergence at the 1 and 1½-inch depths, which averaged 1.2 and 2.5 days, respectively, compared with ½ inch, were not as great. Again, alfalfa was fastest and timothy slowest in emergence with trefoil and bromegrass intermediate. Differences among species and depths in rate of emergence were significant (1% level), as was the interaction of species with depths (1% level). The latter interaction was due mainly to a greater delay in emergence at deeper plantings with trefoil and timothy than with bromegrass. Compared with the check and PR-51, Krilium hastened emergence slightly but significantly (5% level).

Percentages of emergence declined from 79.2% on the average at ½ inch to 72.2% at 1 inch and 58.3% at 1½ inches. Though this decrease

Table 3. Mean number of days to emergence and percentage of emergence for four forage species planted at three depths in untreated, Krilium-treated, and PR-51 treated soil in the greenhouse.

Species	½-inch depth			1-inch depth			1½-inch depth			Species mean
	Check	Krilium	PR-51	Check	Krilium	PR-51	Check	Krilium	PR-51	
					Days to emergence					
Bromegrass	6.0	5.8	6.0	6.5	6.2	6.2	7.2	6.7	6.8	6.4
Timothy	6.8	6.2	6.8	8.5	7.5	8.5	10.2	8.8	10.5	8.2
Alfalfa	3.0	3.0	3.0	4.8	4.0	4.8	5.2	5.0	5.0	4.2
Trefoil	5.2	5.0	5.0	6.5	5.5	6.5	7.8	8.5	9.2	6.6
Treatment mean	5.2	5.0	5.2	6.6	5.8	6.5	7.6	7.2	7.9	
Depth mean		5.1			6.3			7.6		
					Percentage of emergence*					
Bromegrass	74.8	72.2	70.2	71.5	70.5	69.5	69.5	67.2	68.5	70.4
Timothy	81.2	83.2	80.8	55.5	70.5	52.2	32.5	45.0	24.2	58.4
Alfalfa	90.8	91.0	88.5	87.8	90.5	85.0	87.2	86.8	85.5	88.1
Trefoil	71.8	73.8	72.8	71.2	72.2	69.8	51.8	45.2	36.5	62.8
Treatment mean	79.6	80.1	78.1	71.5	75.9	69.1	60.2	61.1	53.7	
Depth mean		79.2			72.2			58.3		

*Final reading 22 days after planting.

was significant (1% level), it was not nearly as great at the $1\frac{1}{2}$-inch depth as in Experiment I. Average stands for species also were higher than in the first test and species differences and the interaction of species with depths were significant (1% level). Alfalfa and bromegrass were highest and second, respectively, in total emergence and showed only minor decreases in stand with deeper planting. Timothy was lowest in total emergence and exhibited a marked decline with deeper planting. Trefoil stands held up well at 1 inch but dropped appreciably at $1\frac{1}{2}$ inches. Average soil treatment effects on stand were negligible, but a significant interaction (5% level) of treatments with species was apparent. Both Krilium and PR-51 reduced stands of trefoil somewhat at the $1\frac{1}{2}$-inch depth. With timothy, Krilium improved stands at greater depths while PR-51 decreased them, especially at $1\frac{1}{2}$ inches. Response to Krilium, therefore, varied in some respects from the first to second experiment.

Experiment III

In this experiment all species, depths and soil treatments studied in the first two tests were incorporated into a field planting in late April. Number of days for emergence were recorded and mean results are given in Table 4. Unlike in the greenhouse, emergence at the 1-inch depth was as rapid as at $\frac{1}{2}$ inch for all species. A significant (1% level) but rather slight delay in emergence was found at the greatest depth. Species also differed significantly (1% level) in mean emergence rate with legumes coming up faster than grasses. Response to depth varied among species, however, as bromegrass emerged as fast at $1\frac{1}{2}$ inches as at $\frac{1}{2}$ inch, while trefoil showed a mean delay of over 3 days. This differential resulted in a significant (1% level) species x depths interaction. PR-51 hastened emergence slightly compared with no treatment while Krilium delayed it, and soil treatment effects varied with species.

Stand counts in the field were not comparable among species as seed size differed and the same number of seeds were not planted per plot. Within each species, however, the same planter setting was used for all depths and soil treatments. Table 5 gives the mean number of seedlings which emerged per 3 feet of row for each variable studied. On the average, stands decreased 19% from $\frac{1}{2}$ to 1 inch and 68% from $\frac{1}{2}$ to $1\frac{1}{2}$ inches. These decreases were highly significant (1% level). Species responded differently to the deeper plantings with trefoil and timothy showing the greatest decrease in stand at $1\frac{1}{2}$ inches and bromegrass the least compared with $\frac{1}{2}$ inch. This interaction of species with depths was significant (1% level) and was similar to that noted in greenhouse experiments. The over-all effect of Krilium was a reduction in stand compared with untreated soil while PR-51 increased average stands at 1 and $1\frac{1}{2}$-inch depths. Soil treatment means differed significantly (1% level) and stand response to treatments varied among species. Soil treatment with PR-51 resulted in less reduction in stand than the check at the 1 and $1\frac{1}{2}$-inch depths in eight out of ten comparisons. Alfalfa showed no improvement in emergence due to PR-51 treatment at either depth. Krilium treatment improved stands of bromegrass at 1 inch and red clover at $1\frac{1}{2}$ inches relative to the check but decreased emergence of

Table 4. Mean number of days to emergence for five forage species planted at three depths in untreated, Krilium-treated, and PR-51-treated soil in the field.

Species	½-inch depth			1-inch depth			1½-inch depth			Species mean
	Check	Krilium	Pr-51	Check	Krilium	Pr-51	Check	Krilium	PR-51	
Bromegrass	16.5	16.5	16.0	16.0	16.2	15.0	16.0	16.8	16.2	16.1
Timothy	18.0	18.0	16.8	16.5	17.0	15.8	19.0	19.8	19.0	17.8
Alfalfa	12.2	12.0	12.0	12.2	12.5	11.8	13.0	13.2	13.2	12.5
Red Clover	12.8	13.0	13.0	12.0	13.0	12.5	14.2	14.5	13.8	13.2
Trefoil	13.0	13.2	13.0	13.0	14.5	13.0	16.2	19.0	15.8	14.5
Treatment mean	14.5	14.6	14.2	14.0	14.6	13.6	15.7	16.6	15.6	
Depth mean		14.4			14.1			16.0		

Table 5. Mean number of seedlings emerged per three linear feet of row for five forage species planted at three depths in untreated, Krilium-treated, and PR-51-treated soil in the field.[1]

Species	½-inch depth			1-inch depth			1½-inch depth			Species mean
	Check	Krilium	PR-51	Check	Krilium	PR-51	Check	Krilium	PR-51	
Bromegrass	25.8	22.2	25.2	15.8	20.2	24.5	17.2	16.5	17.8	20.6
Timothy	63.5	41.5	80.5	46.8	44.2	65.2	11.0	6.5	19.8	32.1
Alfalfa	49.2	57.8	49.5	45.5	36.5	40.0	25.2	22.2	23.8	38.9
Red clover	115.2	84.5	97.5	95.0	71.8	98.5	39.8	47.8	57.0	78.6
Trefoil	121.2	114.8	116.2	86.8	58.8	109.2	16.5	8.0	16.5	72.0
Treatment mean	75.0	64.2	73.8	58.0	46.3	67.5	22.0	20.2	27.0	
Depth mean		71.0			57.2			23.0		

[1]Final reading 36 days after planting.

Table 6. Mean stand and unadjusted and adjusted seedling forage yields for five forage species at the $\frac{1}{2}$-inch planting depth in the field as an average of three soil treatments.

Species	Stand[1]	Seedling forage yield	Adjusted seedling forage yield
Bromegrass	24.4	21.1	34.6
Timothy	61.8	4.8	7.5
Alfalfa	52.2	31.3	36.7
Red clover	99.1	64.6	56.5
Trefoil	117.4	36.5	23.1
Mean	71.0	31.7	31.7

[1] Seedlings per 3 linear feet of row.

[2] Grams per plot of above-ground parts 75 days after planting.

trefoil at all depths and of timothy and red clover at $\frac{1}{2}$ inch. Thus, field response to these soil conditioners was substantially different to that found in the greenhouse.

An evaluation of seedling vigor for all species and soil treatments at the $\frac{1}{2}$-inch planting depth was obtained by harvesting all above-ground parts of each plot 75 days after planting. Weeds were permitted to grow in all plots to provide competition similar to a companion crop and removed just prior to harvest. Mean seedling forage yields were determined on a dry weight basis first and then adjusted for differences in stand among species by use of a covariance analysis. Mean stands and seedling vigor forage yields are presented in Table 6. Even after adjustment for stand differences, species differed significantly (1% level) in seedling vigor. Red clover was best and timothy poorest in vigor of seedling growth while bromegrass and alfalfa were intermediate. Trefoil was below average but still much better than timothy. Unadjusted soil treatment means were 34.2, 32.7, and 28.0 grams per plot for PR-51, untreated and Krilium, respectively. Though treatment differences were not significant statistically for either adjusted or unadjusted yields, the reduction in vigor due to Krilium partly reflected its depressing effect on stand at the $\frac{1}{2}$-inch depth.

Experiment IV

The fourth experiment consisted of five varieties of bromegrass seeded at three depths in Krilium-treated and untreated soil in the field. As shown in Table 7, differences in days for emergence were relatively small for the factors studied. Seedings at the 1-inch depth emerged significantly (1% level) faster than at $\frac{1}{2}$ or $1\frac{1}{2}$ inches, averaging about 1 day earlier. Also, rate of emergence was as fast at $1\frac{1}{2}$ inches as at $\frac{1}{2}$ inch which substantiates results with bromegrass in Experiment III. Varietal differences in days for emergence were slight with a mean range

Table 7. Mean number of days to emergence and seedlings emerged per 3 linear feet of row for five strains of smooth bromegrass planted at three depths in untreated and Krilium-treated soil in the field.

Strain	½-inch depth		1-inch depth		1½-inch depth		Variety mean
	Check	Krilium	Check	Krilium	Check	Krilium	
			Days to emergence				
Canadian Northern	17.0	17.2	16.0	15.8	16.5	16.0	16.4
Southland	17.2	17.2	16.5	16.8	17.2	17.5	17.1
Fischer	17.0	16.5	15.8	15.8	17.2	17.0	16.5
Lincoln	17.0	17.8	16.2	15.8	17.2	17.8	17.0
BR-3	17.2	16.5	15.8	15.8	16.5	16.8	16.4
Treatment mean	17.1	17.1	16.0	16.1	17.0	17.0	
Depth mean	17.1		16.0		17.0		
			Number of seedlings[1]				
Canadian Northern	38.2	41.2	32.8	41.8	30.5	36.8	36.9
Southland	26.5	36.8	22.2	22.5	34.8	28.2	28.5
Fischer	32.8	31.8	21.8	16.2	16.8	17.5	22.8
Lincoln	27.8	18.5	24.0	25.8	16.8	13.0	21.0
BR-3	20.8	35.8	28.2	29.5	30.2	29.8	29.0
Treatment mean	29.2	32.8	25.8	27.2	25.8	25.1	
Depth mean	31.0		26.5		25.4		

[1]Final readings taken 32 days after planting.

of only 0.7 days, though means differed significantly (1% level). Treatment with Krilium apparently had no effect on this trait.

Number of seedlings per 3 linear feet of row (Table 7) varied significantly (1% level) among varieties, ranging from 21.0 to 36.9. Though these differences probably represent variation in germination and seeding rate for the most part, it was interesting to note that the two largest seeded entries, Canadian and BR-3, also were highest in stand count. Decreases in stand from $\frac{1}{2}$ to 1 inch and from $\frac{1}{2}$ to $1\frac{1}{2}$ inches were not significant and averaged 14.5 and 18%, respectively. However, responses to deeper planting varied considerably among varieties. Compared with the $\frac{1}{2}$-inch depth, stands of Fischer decreased substantially at 1 and $1\frac{1}{2}$ inches. Average stands for BR-3 and Southland, on the other hand, were as high or higher at $1\frac{1}{2}$ inches as they were at $\frac{1}{2}$ inch. Mean stand for Krilium-treated soil was 28.4 seedlings compared with 26.6 for untreated soil. This difference was not significant statistically. Response to Krilium treatment among varieties was highly erratic, with unfavorable effects about as common as favorable ones.

Table 8. Mean stand and unadjusted and adjusted seedling forage yields for five varieties of smooth bromegrass at the $\frac{1}{2}$-inch planting depth in the field as an average of two soil treatments.

Strain	Stand[1]	Seedling forage yield[2]	Adjusted seedling forage yield
Canadian Northern	39.8	19.0	14.3
Southland	31.6	15.1	14.8
Fischer	32.2	21.4	20.8
Lincoln	23.1	10.9	15.1
BR-3	28.2	18.1	19.6
Mean	31.0	16.9	16.9

[1]Seedlings per 3 linear feet of row.
[2]Grams per plot of above-ground parts 75 days after planting.

As in Experiment III, seedling vigor measurements were taken on all plots seeded $\frac{1}{2}$ inch deep. Even after adjustment for stand differences, mean seedling forage yields varied significantly (1% level) among varieties. Table 8 gives mean stand and seedling vigor results for the five varieties. Fischer and BR-3, both Iowa strains, were highest in adjusted seedling forage yield, while Canadian was lowest. It is apparent from these results that genotypic differences in early seedling growth exist as well as differences in rate and amount of emergence. Soil treatment effects on seedling forage yield were not significant. Unadjusted mean yields were 17.2 and 16.6 grams per plot for check and Krilium, respectively, indicating no beneficial effect of Krilium on vigor of seedling growth after emergence.

Experiment V

On the basis of results in Experiment IV, which indicated both genetic and seed size effects on planting depth responses in bromegrass, a more extensive study of these factors and their possible interrelationships was undertaken. Experiment V involved 12 entries differing widely in seed size (see Table 9) and four planting depths in a September-planted field test. Data on rate and amount of emergence and on vigor of seedling growth were recorded for all depths and subjected to statistical analyses. Mean days for emergence for the $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2-inch depths were 10.2, 10.1, 10.7, and 12.6 days, respectively. These differences were significant (1% level) due primarily to the delay at the 2-inch depth. Entries also differed significantly (1% level) in rate of emergence, ranging from 9.8 days for Canadian as an average of all depths, to 12.3 days for Southland. Entries responded differentially in rate to depth of planting, as indicated in Table 9 and by a significant (1% level) entries x depths interaction. Smaller seeded entries generally were delayed more in emergence by deeper planting than larger seeded entries.

Stand counts varied among entries due to differences in seed size, planting rate, and germination. The correlation of laboratory germination in sand to stand count was 0.45, but not significant. Analyses of variance indicated significant (1% level) mean differences among depths and among strains in number of seedlings per 4 feet of row. Mean stands for $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2-inch depths were 64.1, 63.4, 56.2, and 37.8 seedlings, respectively. The largest decrease in stand occurred between $1\frac{1}{2}$ and 2 inches, and represented an average loss of 40% compared with the $\frac{1}{2}$-inch depth. Some strains gave better stands at the $1\frac{1}{2}$-inch depth than at $\frac{1}{2}$ inch. For example, BR-3 showed stand increases of 48.0, 63.9, and 14.7% at the 1, $1\frac{1}{2}$, and 2-inch depths compared with $\frac{1}{2}$ inch. This confirms results with this synthetic strain in Experiment IV. Other entries showed progressively greater stand decreases with each greater depth. This interaction between entries and depths in regard to stand establishment clearly shows that some entries are capable of giving better stands at deeper depths than others. Also, as shown in Table 9, most larger seeded entries generally showed less stand reduction with deeper planting than most smaller seeded entries.

Soil moisture conditions were not favorable for seedling growth following planting of this experiment, being dry for most of the period until harvest of seedling top growth 60 days after planting. Despite this, wide and significant (1% level) differences in seedling vigor were noted. As shown in Table 10, mean dry weights of 25 seedlings for all depths varied from 122 to 241 milligrams. Average seedling vigor for the first three depths was almost identical but dropped about 28% for the 2-inch depth. Some grasshopper damage to one replicate of a $\frac{1}{2}$-inch whole plot may have reduced depth differences in seedling vigor. Seedling vigor for five strains was better at $1\frac{1}{2}$ inches than at either $\frac{1}{2}$ or 1 inch. Four of these were the largest in seed size. Topcrosses 129-12, 247-28 (smallest seeded entries) and 502-90, conversely, showed steady decreases in seedling vigor as planting depth increased. Generally, as illustrated in Table 10, the smaller seeded entries showed a greater percentage decrease in top growth at $1\frac{1}{2}$ and 2-inch depths compared with $\frac{1}{2}$ and 1 inch than the larger seeded entries.

Table 9. Mean seed weights, germination percentages, days to emergence and seedlings per 4 feet of row for 12 bromegrass seed entries planted at two depths in the field.

Pedigree	Grams per 300 seeds	Germination (%)	Days for emergence		Seedlings per 4 feet of row[1]		
			1 inch	2 inches	1 inch	2 inches	Decrease (%)[2]
109-6	1.24	93.5	10.0	11.5	47.7	28.0	41
115-5	1.23	89.0	10.0	12.5	43.0	30.7	29
Canadian	1.18	91.0	9.7	11.0	89.0	74.5	16
BR-3	1.12	92.0	10.0	10.7	68.5	53.4	23
502-90	1.09	89.0	9.7	12.5	59.2	52.0	12
261-36	1.06	93.0	10.0	13.0	40.0	21.7	46
Fischer	0.93	81.0	10.0	12.0	60.5	25.7	58
Southland	0.90	49.5	11.2	14.7	40.5	17.7	56
166-40	0.88	93.0	10.7	12.7	61.2	28.7	53
149-21	0.84	91.0	10.2	12.5	96.2	64.2	33
129-12	0.62	90.5	9.7	13.7	88.2	30.5	65
247-28	0.61	96.0	10.0	15.2	66.7	27.5	59
Mean			10.1	12.6	63.4	37.8	40

[1] Final reading 25 days after planting. Planting rate not adjusted for germination or seed size so stands not comparable between entries.

[2] Per cent decrease from 1 to 2-inch depth of seeding.

Table 10. Mean seed weights and seedling forage yields for 12 bromegrass seed entries planted at four depths in the field.

Pedigree	Grams per 300 seeds	Milligrams per 25 seedlings[1]		Seedling weights in per cent of $\frac{1}{2}$-inch depth		
		$\frac{1}{2}$-inch depth	Mean of all depths	1 inch	$1\frac{1}{2}$ inches	2 inches
109-6	1.24	182	203	120	137	90
115-5	1.23	208	230	121	132	89
Canadian	1.18	221	208	101	108	68
BR-3	1.12	139	170	114	139	104
509-90	1.09	263	241	103	92	72
261-36	1.06	265	226	93	94	54
Fischer	0.93	202	195	97	110	79
Southland	0.90	162	133	85	80	65
166 40	0.88	196	164	77	95	62
149-21	0.84	174	152	100	76	73
129-12	0.62	169	135	87	79	52
247-28	0.61	142	122	94	87	64
Mean		194	182	101	102	72

[1] Oven-dry weights of above-ground parts 60 days after seeding.

Experiment VI

This experiment was an exact duplicate of Experiment V except that it was conducted in the greenhouse. The main objective was to determine degree of relationship between greenhouse and field responses to genetic, seed size, and depth factors and their effects on seedling vigor traits. The test was planted in a cool greenhouse in late November and soil moisture conditions were kept favorable for germination and growth. Number of days for emergence were considerable more than in previous greenhouse tests with bromegrass, averaging 16.4, 20.3, 26.4, and 28.6 days for $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2-inch depths, respectively. This steady increase in emergence time also differed from field results where the first three depths were very similar. For all depths, average emergence time ranged from 20.2 to 27.3 days among the 12 entries. Both depth and strain effects were highly significant (1% level). However, as shown in Table 11, days for emergence were about the same for all entries at $\frac{1}{2}$ inch, except for Southland which had poor seed quality and low germination. The delay in emergence at 2 inches compared with $\frac{1}{2}$ inch generally was greatest for the smallest seeded entries and least for the largest. As examples, Topcross 109-6 with the largest seed showed a delay of only 8.5 days compared with the small seeded entry, Topcross 129-12, which was delayed 15.5 days. Despite this obvious differential response, the entries x depths interaction for days to emergence was not statistically significant.

Stand counts in this test were based on a planting rate of 50 seeds per plot and showed a highly significant (1% level) correlation of 0.83 with germination percentage (see Table 9). The relationship was best at the $\frac{1}{2}$-inch depth where all but Southland and Fischer gave very good germination. As shown in Table 11, emergence percentage decreased with deeper plantings, with average decreases of 10%, 28%, and 37% for the 1, $1\frac{1}{2}$, and 2-inch depths, respectively, compared with $\frac{1}{2}$ inch. An analysis of variance indicated significant (1% level) differences among depth and entry means and a significant (1% level) interaction of entries with depths. Larger seeded entries were higher in mean stand than smaller seeded entries. This difference was noticeable at the 1-inch depth and the differential tended to increase with depth. At the $1\frac{1}{2}$ and 2-inch depths, the three smallest seeded entries showed less than 53% emergence, while the three largest seeded entries all exceeded 70%.

Seedling vigor differences were equally pronounced. Unlike in the field, however, greater decreases in seedling vigor occurred with each deeper depth. Mean reductions in seedling weight were 27, 47, and 51% for the 1, $1\frac{1}{2}$, and 2-inch depths, respectively, compared with $\frac{1}{2}$ inch. In the field average vigor was similar for the first three depths and dropped 28% at 2 inches. As indicated in Table 12, entries differed significantly (1% level) in mean vigor at all depths with larger seeded entries generally giving the heaviest seedlings. There was a tendency for less reduction in seedling vigor as depth of seeding increased among the larger seeded entries compared with smaller seeded lots, but the statistical interaction of depths x entries was not significant. Nevertheless, some large seeded entries produced as vigorous seedlings at $1\frac{1}{2}$ and 2-inch depths as small seeded entries did at $\frac{1}{2}$ inch.

Table 11. Mean seed weights, days for emergence, and emergence percentages for four planting depths for 12 bromegrass seed entries in the greenhouse.

Pedigree	Grams per 300 seeds	Days for emergence		Total emergence (%)			
		½ inch	2 inches	½ inch	1 inch	1½ inch	2 inches
109-6	1.24	15.5	24.0	89.6	90.6	82.6	71.6
115-5	1.23	16.0	26.0	90.6	88.6	70.6	71.0
Canadian	1.18	16.5	27.0	92.6	87.6	78.6	69.6
BR-3	1.12	15.8	28.0	92.6	86.6	77.6	61.6
502-90	1.09	16.0	28.0	93.0	90.0	73.6	67.0
261-36	1.06	15.3	26.8	97.6	87.6	78.6	80.0
Fischer	0.93	16.8	28.5	78.6	67.6	52.6	48.6
Southland	0.90	19.5	33.8	49.6	39.0	29.6	19.6
166-40	0.88	16.3	29.3	90.0	81.6	66.0	37.6
149-21	0.84	16.8	29.8	94.6	78.0	49.0	52.6
129-12	0.62	15.8	31.3	93.0	76.6	46.6	36.6
247-28	0.61	16.5	30.3	90.6	78.0	52.6	49.0
Mean		16.4	28.6	87.8	79.4	63.2	55.4

Table 12. Mean seed weights and seedling forage yields for 12 bromegrass seed entries planted at four depths in the greenhouse.

Pedigree	Grams per 300 seeds	Milligrams per 20 seedlings[1]		Seedling weights in per cent of ½-inch depth		
		½-inch depth	Mean of all depths	1 inch	1½ inches	2 inches
109-6	1.24	102	74	72	64	54
115-5	1.23	109	82	73	64	62
Canadian	1.18	96	70	78	56	56
BR-3	1.12	85	60	70	59	54
502-90	1.09	98	65	71	48	48
261-36	1.06	101	71	70	55	53
Fischer	0.93	80	54	76	46	49
Southland	0.90	55	35	76	42	34
166-40	0.88	75	49	66	52	44
149-21	0.84	59	40	77	49	47
129-12	0.62	58	34	58	40	32
247-28	0.61	62	41	75	39	46
Mean		81	56	73	53	49

[1] Seedling vigor basedon dry weight of above-ground parts of 20 seedlings per plot harvested 49 days after planting.

Greenhouse and Field Relationships

One major objective of the present study was to relate seedling vigor responses attained in the greenhouse with that in the field. Correlations for rate and amount of emergence in Experiments I and II in the greenhouse with field results in Experiment III are presented in Table 13. It is apparent that relative differences in days for emergence among species at different planting depths and subjected to different soil conditioner treatments were similar in the greenhouse and field. Gross correlations were highly significant (1% level) for both comparisons, while correlations for individual depths and treatments for rate of emergence all were above 0.82 except for one. Associations for total emergence were low in most instances and all negative at the $\frac{1}{2}$-inch depth. Appreciable and positive relationships of greenhouse and field emergence were noted only at the $1\frac{1}{2}$-inch depth, where two of five correlations were significant (5 and 1% level). The gross correlation for total emergence between Experiments I and III was significant (5% level) and positive but of low predictive value. Part of these low relationships for total emergence may be explained by failure to plant uniform numbers of seeds for all species in the field. However, differential responses among species to test conditions probably also were involved.

Relation of greenhouse and field results for rate of emergence and seedling vigor in Experiment V in the field and Experiment VI in the greenhouse are illustrated in Table 14. Correlations for all depths combined and for the 1-inch depth and deeper were all significant (1% level except one at 5% level) and positive for both traits. Almost no correlation for days to emergence at $\frac{1}{2}$ inch was observed while that for seedling vigor was significant (5% level). Because of the wide discrepancy of seeding rates in the field due to differences in seed size among entries, no correlations were computed for total emergence. However, it appears that a fairly good association between greenhouse and field prevails for distinguishing relative strain differences in seedling vigor at 1 and $1\frac{1}{2}$-inch depths of seeding.

Seed Weight Relationships

As already indicated by data from Experiments V and VI, seed weight in bromegrass had a pronounced effect on seedling vigor attributes. Correlations between seed weight and both days for emergence and seedling vigor, given in Table 15, confirm these observations. All associations for seedling vigor and weight were highly significant (1% level) except for the $\frac{1}{2}$-inch depth in the field. The latter relationship may have been biased by grasshopper damage. The degree of association tended to be somewhat higher in the greenhouse.

Correlations between seed weight and days for emergence were generally negative and highest at the greatest depths. At the $\frac{1}{2}$ and 1-inch depths, relationships were variable and low in predictive value. Again, correlations were higher in the greenhouse than in the field. These results suggest that strain differences in emergence and seedling vigor might be evaluated in the greenhouse at planting depths below 1 inch. Such a procedure would eliminate problems of soil moisture availability, weeds, and planting precision which occur in field tests.

Table 13. Correlation coefficients for rate and amount of emergence in the greenhouse with that in field experiments for different planting depths and soil treatments. (Experiments I, II, and III).

Treatment	Depth of planting (inches)	Experiments I and III			Experiments II and III		
		D.F.	Days for emergence	Total emergence	D.F.	Days for emergence	Total emergence
No treatment	½	3	0.91*	-0.32	2	0.89	-0.42
	1	3	0.77	-0.04	2	0.82	0.03
	1½	3	0.84	0.56	2	0.99**	0.96*
Krilium	½	3	0.90*	-0.08	2	0.90	-0.12
	1	3	0.85	0.16	2	0.98*	-0.08
	1½	3	0.86	0.61	2	0.99**	0.99**
PR-51	½	-	--	--	2	0.94	-0.08
	1	-	--	--	2	0.84	0.26
	1½	-	--	--	2	0.88	0.57
Gross correlation		28	0.74**	0.44*	34	0.82**	0.29

* Exceeds 5% level of probability.
** Exceeds 1% level of probability.

Table 14. Correlation of field and greenhouse results for days to emergence and seedling vigor of 12 bromegrass entries at four planting depths. (Experiments V and VI).

Depth of planting	D.F.	Days for emergence	Seedling vigor
0.5	10	-0.01	0.65*
1.0	10	0.77**	0.89**
1.5	10	0.71**	0.93**
2.0	10	0.69*	0.82**
Gross correlation	46	0.70**	0.91**

* Exceeds 5% level of probability.
** Exceeds 1% level of probability.

Table 15. Correlations of seed weight with mean days for emergence and seedling vigor in field and greenhouse tests of 12 bromegrass entries planted at four depths (Experiments V and VI).

Items correlated	Planting depth (inches)	"r" value	
		Field	Greenhouse
Seed weight and days for emergence	0.5	0.37	-0.24
	1.0	-0.17	-0.51
	1.5	-0.45	-0.70**
	2.0	-0.73**	-0.76**
	Mean of all depths	-0.49	-0.66*
Seed weight and seedling vigor	0.5	0.44	0.87**
	1.0	0.79**	0.90**
	1.5	0.85**	0.91**
	2.0	0.85**	0.87**
	Mean of all depths	0.80**	0.89**

* Exceeds 5% level of probability for 10 D.F.
** Exceeds 1% level of probability for 10 D.F.

DISCUSSION

In the present study the effects of several cultural factors on seedling vigor traits of bromegrass and other important species were investigated in a series of experiments. Results in certain instances agree with previous findings but in others new interpretations appear possible. Observations and findings on soil conditioner effects, differential responses of species and strains of bromegrass to planting depth, relationships of greenhouse and field performance and significance of seed weight in bromegrass all provided valuable new information pertaining to stand establishment problems. Also furnished were valuable suggestions for further research in this field.

Responses to soil conditioner treatments were variable. In the first experiment in the greenhouse Krilium improved emergence of all species at 1 and 1½-inch depths of seeding with no appreciable effect on rate of emergence. In the second experiment, also planted in the greenhouse using the same soil but with higher temperatures prevailing, it hastened average emergence slightly at all depths but had a negligible effect on stand in most instances. Actually, Krilium reduced stands of trefoil somewhat at 1½ inches and improved timothy stands substantially at 1 and 1½ inches. In the field Krilium generally delayed emergence in Experiment III but not in Experiment IV and decreased stands of most species in the former test at ½ and 1 inch. In all tests soil treatment with Krilium hastened drying out of the soil; and this explained in part its adverse effects in the field where soil moisture near the surface was variable in the period subsequent to planting. Seedling weights in both field tests were somewhat less with Krilium than on check plots. Thus, use of soil conditioners of the nature of Krilium apparently shows little promise for improvement of stand establishment under field conditions of the species studied herein. It is possible, however, that under specialized conditions of heavy and poor soil structure and cool, wet conditions such conditioners may be used beneficially for forage or turf establishment, as indicated by results in Experiment I in the greenhouse.

PR-51, a surfactant or wetting agent, gave different results in the greenhouse and field. In the greenhouse it tended to reduce stands, particularly for timothy and trefoil at 1½ inches, with no effect on rate of emergence. In the field PR-51 hastened emergence somewhat and improved stands of most species at the 1 and 1½-inch depths. It also slightly improved seedling vigor compared with the check. Because of low application rates, these results suggest that soil conditioners of this nature merit additional study under field conditions for possible beneficial use in establishment of small field seeds.

Planting depth results in the first three experiments generally agree with previous studies of this factor (28, 29, 32). Legumes emerged more rapidly than grasses under both greenhouse and field conditions with alfalfa fastest and timothy slowest. Depth responses in rate of emergence, however, were variable among species and between greenhouse and field. In the greenhouse all species showed a delay in emergence as planting depth increased, particularly timothy, while bromegrass showed the least response. In the field emergence was as fast or faster at 1 inch

than at $\frac{1}{2}$ inch, even for timothy. Bromegrass emerged as fast in most instances at $1\frac{1}{2}$ inches as at shallow depths, while other species all were delayed to some extent when planted deeper than 1 inch.

Under greenhouse conditions stand counts decreased appreciably with deeper planting and timothy showed the greatest decreases. Total emergence for bromegrass, alfalfa and red clover dropped only slightly from $\frac{1}{2}$ to 1 inch in both tests and stands were relatively high at $1\frac{1}{2}$ inches in the second test. Surprisingly, trefoil came up very well at 1 inch in the second test. Since one of these tests was planted in January and one in March, temperatures and sunlight were more beneficial in the second test and resulted in improved emergence at 1 and $1\frac{1}{2}$ inches. In the field total emergence also decreased with depth with greatest reductions occurring for timothy and trefoil and least for brome grass at the $1\frac{1}{2}$-inch depth. Relative emergence for timothy at 1 inch compared with $\frac{1}{2}$ inch seeding was better than trefoil, similar to bromegrass, alfalfa and red clover, and reasonably good. This was contrary to greenhouse results where trefoil emerged relatively better than timothy at 1 and $1\frac{1}{2}$ inches. In agreement with previous results, present data do indicate best emergence from the $\frac{1}{2}$-inch depth under optimum conditions for the species studied. They also point out why timothy and trefoil are less able to establish stands under variable planting depths than the other species. However, indications are that under field conditions where moisture usually is variable, emergence may be as good and faster at 1 inch than at $\frac{1}{2}$ inch.

Of all species studied in the first three experiments bromegrass generally showed the least response to planting depth. Results in the last three experiments illustrated two other very important factors in this response, namely, seed weight and genotype. It was apparent that strains of bromegrass differ in ability to emerge and in rate of emergence from different depths of planting. Moreover, seed weight has a marked effect on seedling vigor attributes with larger seeds giving most favorable responses, particularly at depths of seeding greater than 1 inch. These findings relative to genetic and seed weight effects agree with other forage investigations (4, 16, 21, 22, 24, 29, 33, and 35) on similar factors and point out the need for their consideration in any subsequent studies on seedling vigor and stand establishment. They also indicate that breeding for improved seedling vigor and seed weight in bromegrass would serve the same objective of increasing seedling vigor. Larger seeded strains, in addition, would facilitate harvesting, cleaning and planting ease with this species and possibly enable more precise and deeper planting by drilling.

Another seedling vigor factor indicated by bromegrass studies was that of seed quality. The seed lot of Southland used in Experiment IV was good in germination and emerged as fast and better at $1\frac{1}{2}$ inches than at $\frac{1}{2}$ inch in untreated soil. The lot used in Experiments V and VI was different and poor in germination. In these tests Southland showed marked decreases in rate and amount of emergence with deeper planting and was much poorer in seedling vigor than other strains of the same seed weight. Such results substantiate other observations (8, 13, 17, 27) that age and condition of seed can markedly influence seedling vigor expressions of a species or a strain within a species. Ideally then, seedling

vigor studies which compare strains or possibly even species should use only uniform, high quality seed lots produced under comparable conditions in the same year and location to eliminate such confounding effects. Undoubtedly, many past studies did not consider this factor in stand establishment studies, and as a consequence, misleading results were obtained.

Since bromegrass was common to all experiments it is possible to compare seasonal and location effects on seedling vigor attributes. Combined results for days to emergence at three planting depths, using data from the variety Fischer, for experiments IV, V, and VI, are as follows:

Location	Time of planting	Experiment No.	½"	1"	1½"
Greenhouse	November	VI	16.8	19.8	28.8
	January	I	6.0	7.5	9.8
	March	II	6.0	6.5	7.2
Field	April	III	16.5	16.0	16.0
	April	IV	17.0	15.8	17.2
	September	V	9.7	10.0	10.5

It is obvious that season of planting has a marked effect on emergence rate in both greenhouse and field, due in large part to variations in temperature and sunlight. Rate of emergence varied over 10 days at ½ inch and over 21 days at 1½ inches in the greenhouse at different seasons. The range was over 6 days in the field. Deductions as to average days for emergence in bromegrass would be difficult on the basis of greenhouse results herein. Similar comparisons for percentage of emergence and seedling forage yield also indicate the variable influence of the several environmental factors on performance and signify the need for uniform environmental controls in precise comparisons of species and strains in seedling stages.

Evaluation of seedling weights in the early growth stages provided explanations for several common field observations with species and strains studied. Timothy seedlings were very weak which helps explain the poor aggressiveness of this species (7) in spring plantings. Trefoil actually germinated well and grew fairly vigorously in the field the first few weeks after planting before weed competition became a factor. Apparently, stand establishment problems with this species are not due nearly as much to germination and emergence aspects of seedling vigor *per se* as to weak competitive ability of seedlings with weeds and companion crops and to slow seedling growth (12). The superior seedling vigor observed for red clover certainly points out the good competitive ability of this species and usually good stand establishment under varying conditions in spring plantings (6, 12). Seedling vigor of bromegrass approached that of alfalfa in Experiment III, which illustrates its establishment capacities. There also was evidence in the last three studies that bromegrass strains differ in seedling vigor aside from seed weight influences, as noted by Tossell (35). Additional information on this point will be presented in another paper in regard to improvement by breeding for better seedling vigor.

Comparison of greenhouse and field results of the various experiments revealed better associations at deeper planting depths. This would indicate that the greenhouse, where environmental control is easier than in the field, might be used for preliminary evaluations of seedling vigor attributes of strains and selections providing plantings are made below 1 inch in depth. Two other aspects of seedling vigor not studied herein also would appear important. The first is the relationship of seedling vigor differences among strains to subsequent forage productivity and performance in mixtures (6). The other is the significance of root development in expression of cultural and genetic effects on seedling vigor. Both of these could play a significant role (32) in stand establishment and deserve further investigation.

SUMMARY

1. Planting depth, soil conditioners, species and strain differences, and seed weight were studied as factors in seedling vigor of bromegrass and other forage species in a series of experiments. Association of greenhouse and field responses to these factors was considered as well as their interrelationships. In the various tests, seedling vigor was evaluated in terms of rate and amount of emergence and in weight of seedling growth the first few weeks after emergence.

2. Planting depth proved to be a major factor in the expression of seedling vigor of all species studied. Generally, rate and amount of emergence decreased as planting depth increased from $\frac{1}{2}$ to 1 to $1\frac{1}{2}$ inches, although the relative effects varied among species and seasons of the year and from greenhouse to the field. Bromegrass and alfalfa showed the least decline in emergence and trefoil and timothy the most with deeper planting, while rate of emergence was fastest for alfalfa and slowest for timothy in all experiments. Emergence in all field tests was as rapid as 1 inch as at $\frac{1}{2}$ inch, illustrating a different response than in the greenhouse where $\frac{1}{2}$ inch always was best.

3. Bromegrass strains differed markedly in seedling vigor attributes at various planting depths up to 2 inches. In the field several strains emerged as fast and as good from $1\frac{1}{2}$-inch depths as from $\frac{1}{2}$ and 1 inches. Larger seeded strains generally were superior to small seeded strains in seedling vigor attributes at all depths with superiority tending to increase with depth in both greenhouse and field. Also, some large-seeded strains produced more vigorous seedlings at $1\frac{1}{2}$ inches than some small-seeded strains did at $\frac{1}{2}$ inch in all tests. Evidence of strain differences in seedling vigor aside from seed weight effects also was found, indicating two promising ways for improvement of stand establishment in bromegrass by breeding.

4. Soil treatment with Krilium improved emergence in only one of four tests and that was in the greenhouse. In the field it had little effect in one test with bromegrass and in the other decreased over-all species emergence and rate of emergence at the three depths. In contrast,

PR-51 decreased emergence for trefoil and timothy at greater depths in the greenhouse and increased emergence for most species at greater depths in the field. It also hastened emergence slightly in the latter instance. Neither soil conditioner treatment measurably affected seedling growth rate of any species or strain studied in the field.

5. In one test red clover was outstanding in vigor of seedling growth 75 days after planting in the field. Alfalfa and bromegrass ranked second in this regard with trefoil third and about 25% lower. Timothy was by far the poorest in growth rate with seedling forage yields about $\frac{1}{2}$ of trefoil and 1/8 of red clover.

6. Correlations of greenhouse and field results for rate and amount of emergence and seedling forage yield generally were highest at the greater depths, which suggests that plantings below 1 inch in the greenhouse would give best predictions of field performance. Other suggestions arising from the results in regard to future research in stand establishment included the necessity of using comparable seed lots and environmental conditions for species and strain comparisons, need for attention to root development in seedling stages and study of relationships between seedling vigor attributes and subsequent forage yields and forage mixture composition.

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EVALUATION OF VARIOUS METHODS AND REAGENTS FOR TOTAL HARDNESS AND CALCIUM HARDNESS IN WATER

Charles A. Geotz and Robert C. Smith

Department of Chemistry
Iowa State University, Ames

Abstract

Many new reagents and method modifications for hardness determinations have recently appeared. A critical study and evaluation of these reagents and methods has been performed with special emphasis upon the speed, accuracy, ease of end point detection, and applicability to various types of water samples. Stability of reagents and removal of interfering ions were also noted. The effect of varying pH upon accuracy of the various indicator end points was investigated.

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I. INTRODUCTION

Many modifications of the total hardness and calcium hardness titrations using EDTA have been introduced (1, 4-6, 8-17, 19, 20, 22-46). Two total hardness methods (4, 17, 22) were presented as tentative standard methods in the 10th ed. of "Standard Methods for the Examination of Water, Sewage and Industrial Wastes" (43) in 1955. Several new reagents for, and modifications of, these methods have been introduced (8, 16, 26, 28, 31, 32, 37, 45).

In view of the many new reagents and method modifications, the authors decided to make a critical study and evaluation.

II. REAGENTS

A. Mineral-free Water

Distilled water showing no hardness or interfering ions (particularly copper) or preferably demineralized water (using Amberlite MB-2) was used.

B. Standard Calcium Chloride Solution

1.000 gram primary standard grade calcium carbonate (7) (Mallinckrodt or Hach) was dissolved in about 100 ml of water containing 10 ml of 3 N hydrochloric acid, boiled gently, neutralized with ammonium hydroxide using methyl red as the pH indicator, and diluted to one liter.

C. EDTA Solutions (0.01 M)

Titrant No. 1. EDTA plus magnesium ions (17, 22, 23, 43)

Four grams of reagent grade disodium salt of EDTA and 0.1 gram of magnesium chloride hexahydrate were dissolved in 750 ml of ion exchange water and standardized against the standard calcium chloride solution with dilution until 1 ml was nearly equivalent to 1 mg of calcium carbonate. When this titrant and those following were not exactly equivalent to 1 mg of calcium carbonate per ml, an appropriate factor was used.

Titrant No. 2. EDTA plus sodium hydroxide (4, 43)

Four grams of reagent grade EDTA disodium salt and 0.9 gram of sodium hydroxide were dissolved in 800 ml of water and standardized and diluted so that 1 ml was nearly equivalent to 1 mg of calcium carbonate. (The solution was adjusted to pH 10 with sodium hydroxide before final standardization using the borate buffer type procedure.)

Titrant No. 3. EDTA with no magnesium or sodium hydroxide added (24-26, 33)

Four grams of reagent grade EDTA disodium salt were dissolved in about 750 ml of water and standardized and diluted so that 1 ml was nearly equivalent to 1 mg of calcium carbonate. (The buffers used with this titrant needed to contain the magnesium salt of EDTA.)

D. Magnesium EDTA Complex

Magnesium salt of EDTA was obtained commercially and used as indicated (Hach Chemical Co.).

E. Buffers and pH Adjustment Solutions

Buffer No. 1. Ammonium hydroxide-ammonium chloride only (17, 22, 23, 43)

The pH 10 ammonia buffer was prepared by dissolving 67.5 grams of ammonium chloride in 570 ml of concentrated ammonium hydroxide, diluting to 1 liter, and storing in an airtight screw-cap polyethylene bottle.

Buffer No. 2. Ammonium hydroxide-ammonium chloride containing complexed magnesium (24-26, 33)

This ammonia buffer was prepared as Buffer No. 1 except that 5 grams of complexometrically neutral magnesium salt of EDTA were also added.

Buffer No. 3. Odorless nonvolatile buffer containing magnesium (26)

This buffer was obtained commercially ready for use (Hach Chemical Co.).

Buffer No. 4. Monoethanolamine-hydrochloric acid buffer containing complexed magnesium (37)

This buffer was prepared by mixing 300 ml of redistilled monoethanolamine with 55 ml of concentrated hydrochloric acid, adding 5 grams of magnesium salt of EDTA, and diluting to 1 liter.

Buffer No. 5. Borate-sodium hydroxide-sodium sulfide (4, 43)
This buffer was prepared by dissolving 40 grams of sodium tetraborate in 800 ml of water with heating. Ten grams of sodium hydroxide, 10 grams of Rochelle salt, and 10 grams of sodium sulfide were dissolved in 100 ml of water. When both solutions were cool, they were combined and diluted to 1 liter.

Buffer No. 6. MonoVer (26)
Odorless nonvolatile buffer containing complexed magnesium and indicator obtained ready for use (Hach Chemical Co.).

Buffer No. 7. UniVer I Powder (26)
Buffer-Inhibitor-Indicator containing complexed magnesium obtained ready for use (Hach Chemical Co.).

Buffer No. 8. UniVer II Powder (26)
Buffer-Inhibitor-Indicator containing complexed magnesium obtained ready for use (Hach Chemical Co.).

pH Adjustment Solutions. Sodium hydroxide solutions with concentrations of 2 N, 4 N, and 8 N were prepared.

F. Indicators.

1. Total Hardness Indicator Preparations of Eriochrome Black T
   a. Powder forms
      1) A diluted powder indicator mixture was prepared by grinding 0.5 gram of practical analytical reagent grade F-241 powder with 25 grams of potassium sulfate.
      2) A variety of commercially obtained powder mixtures (26) with inhibitor and in some cases buffer properties were used as obtained (Hach Chemical Co.).
         a) ManVer Powder (indicator-inhibitor).
         b) UniVer I Powder (indicator-buffer-inhibitor).
         c) UniVer II Powder (indicator-buffer-inhibitor).
   b. F-241 Indicator Solutions
      1) A methanol solution of the dye was prepared by dissolving 0.2 gram of the practical analytical reagent grade F-241 powder in 50 ml of commercial grade methanol.
      2) Commercially obtained F-241 indicator solutions (26) with inhibitor and buffer were used as obtained (Hach Chemical Co.).
         a) MonoVer (indicator-buffer-inhibitor).
         b) ManVer (indicator-inhibitor).

2. Calcium Hardness Indicator Preparations
   a. Calcium Indicator Powders
      1) Murexide (CalVer I). The diluted indicator powder (26) was obtained commercially (Hach Chemical Co.).
      2) Calcon (Eriochrome Blue Black R). This dye was obtained commercially (J. T. Baker Chemical Co.). A diluted powder indicator was prepared by grinding 0.5 gram of the dye together with 25 grams of potassium sulfate.

3) CalVer II. The diluted powder form indicator (26) was obtained commercially (Hach Chemical Co.).

4) Calcein. This dye was obtained commercially (G. Frederick Smith Chemical Co.). Two powder indicator mixtures were prepared.

a) One gram of calcein dye was ground together with 10 grams of charcoal and 100 grams of potassium chloride (16).

b) A diluted powder mixture of 0.2 gram calcein, 0.12 gram thymolphthalein, and 20 grams potassium chloride were ground together (45).

b. Calcium Indicator Solutions

1) Calcon Indicator Solution. A calcium indicator solution was prepared by dissolving 0.2 gram of the dye in 50 ml of methanol (28).

2) Calcein Indicator Solution. A calcium indicator solution was prepared by dissolving 2 grams of calcein in 25 ml of 1 N sodium hydroxide and diluting to 100 ml with water (16).

G. Inhibitors

1. Sodium Cyanide

a. Powder form, reagent grade.

b. Sodium cyanide solution (Inhibitor a). One gram of sodium cyanide was dissolved in 100 ml of distilled water (43).

2. Sodium Sulfide Inhibitor Solution (Inhibitor b). This solution was prepared by dissolving 5 grams of sodium sulfide ($Na_2S \cdot 9H_2O$) in 100 ml of water (43).

III. APPARATUS

A Beckman Model B pH meter was used for all pH determinations. A Beckman Type E glass electrode was used in pH 11 or higher measurements. Titrations were conducted in 250 ml beakers sitting on white cloth. The room was equipped with daylight fluorescent lamps. Solutions were stirred using magnetic stirrer with teflon-coated stirring bars.

IV. EXPERIMENTAL - TOTAL HARDNESS

A. Introduction

A variety of synthetic "hard waters" were prepared using the primary standard grade calcium carbonate and magnesium ribbon. In some cases only calcium carbonate was used since the absence of the magnesium ion was studied. The general procedure was to dissolve accurately weighed amounts of the materials in about 100 ml of water containing 10 ml of 3 N hydrochloric acid, boil gently to remove carbon dioxide, cool, and neutralize the excess hydrochloric acid with dilute ammonium hydroxide using methyl red as the indicator. The resultant solution was diluted to exactly 1 liter to give a known strength solution. Twenty-five-ml aliquots were taken by pipet and mixed with about 25 ml of demineralized water for each titration.

Table I. Gravimetric Analysis of a Synthetically Prepared Hardwater 845.2 ppm $CaCO_3$ and 57.2 ppm of magnesium expressed as $CaCO_3$. Total Hardness 902.4

Grams CaO weighed	ppm $CaCO_3$ found	Dev. from av. in ppm	Grams $Mg_2P_2O_7$ weighed	Mg as ppm $CaCO_3$ found	Dev. from av. in ppm	Total Hardness	Dev. from av. in ppm
0.1183	844.4	-0.5	0.0159	57.2	0.0	901.6	-0.5
0.1184	845.2	+0.3	0.0165	59.4	+2.2	904.6	+2.5
0.1188	848.0	+3.1	0.0150	54.0	-3.2	902.0	-0.1
0.1185	846.0	+1.1	0.0167	60.0	+2.8	906.0	+3.9
0.1178	840.8	-4.1	0.0154	55.4	-1.8	896.2	-5.9
Average	844.9	1.8		57.2	2.0	902.1	2.6

B. Total Hardness Methods

Method No. 1. Gravimetric Analysis (APHA Standard Method)

One of the synthetic hard waters was analyzed by the standard conventional gravimetric method (43). The results are presented in Table I. The average hardness (in ppm $CaCO_3$) was found to be 902.1 while the hardness as calculated from the primary standard materials was 902.4. This table showed that within reasonable experimental error the method for preparing the standard synthetic water samples was satisfactory. Approximately 24 hours were required to carry out analysis using this method.

Table II. Soap Titration for Total Hardness

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
845.2	57.6	902.8	886.2	-16.6
200.0	618.6	818.6	1259	+440.4
			1145	+326.4
100.0	412.4	512.4	653.1	+140.7
			655.5	+143.1
203.6	111.5	315.1	293.0	-22.1
			274.0	-41.1
100.8	38.3	139.1	134.9	-4.2
			121.8	-17.3
50.8	0.0	50.8	51.4	-0.6
			49.7	-1.1
9.5	0.0	9.5	7.6	-1.9
			9.9	+0.4
			Av. dev.	88.9

Method No. 2. Soap Titration (APHA Standard Method)

Seven synthetic water samples were analyzed by the soap titration method (43) and the data are given in Table II. It was apparent that when the total hardness is high, the method appears to be very nearly useless, especially when considerable magnesium was present. If we knew in advance approximately how hard the water was, about 15 to 20 minutes were required for each titration. When the hardness was unknown, even longer time was required. This time included obtaining an average lather factor which had to be determined.

Table III. EDTA Method—Ammonia Buffer (Tentative Method)

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
845.3	58.4	903.7	905.8	+2.1
845.5	58.0	903.5	903.7	+0.2
845.2	58.1	903.3	903.3	0.0
			903.7	+0.4
845.1	57.9	903.0	902.8	-0.2
845.2	57.6	902.8	902.4	-0.4
845.2	57.2	902.4	902.9	+0.5
845.4	28.8	874.2	874.7	+0.5
			874.8	+0.6
845.3	28.8	874.1	873.5	-0.6
			872.7	-1.4
200.0	618.6	818.6	818.9	+0.3
			816.6	-2.0
			818.2	-0.4
200.0	400.0	600.0	603.0	+3.0
400.0	199.4	599.4	597.1	-2.3
100.0	412.4	512.4	513.2	+0.8
			514.0	+1.6
203.6	111.5	315.1	315.4	+0.3
100.8	38.3	139.1	137.9	-1.2
50.8	0.0	50.8	50.3	-0.5
9.5	0.0	9.5	10.8	-1.3*
			Av. dev.	1.0

* Very poor endpoint

Method No. 3. EDTA Method, Ammonia Buffer (APHA Tentative Method)

Sixteen synthetic waters were analyzed using this method (17, 22, 23, 43), and the data are presented in Table III. This method called for presence of the magnesium ions in the titrant (Titrant No. 1-Buffer No. 1). Satisfactory results were obtained, except in low hardness waters containing little or no magnesium. Here the end point was poor, because not enough titrant had been added to build up a proper level of magnesium in the titration vessel.

Method No. 4. EDTA Method, Borate Buffer (APHA Tentative Method)
The titrant used in this method (4) contained sodium hydroxide (Titrant No. 2-Buffer No. 5), but no magnesium. The pH of the titrant was 10, therefore special storage conditions were necessary, or the strength of the titrant would change rather rapidly according to the study of Loomis (21). The data are shown in Table IV. This method was shown to be less accurate than the other EDTA procedures. When no magnesium was present in the sample being titrated, the end point was so poor compared to the other EDTA methods that we classified it as failing. The buffer failed to control the pH at about 10 at the end point (see Buffer Capacity Section). This occurrence was noted especially in high synthetic hardness waters.

Table IV. EDTA Method—Borate Buffer (Tentative Method)

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
845.2	57.6	902.8	913.3	+10.5
			910.1	+7.2
			908.0	+5.2
100.0	412.4	512.4	508.1	-4.3
			505.2	-7.2
			524.4	+12.0
203.6	111.5	315.1	315.7	+0.6
100.8	38.3	139.1	137.4	-1.7
50.8	0.0	50.8	fails	
9.5	0.0	9.5	fails	
			Av. dev.	6.1

Method No. 5. EDTA Method, No Magnesium in the titrant and Magnesium salt of EDTA in the Ammonia Type Buffer (24-26, 33)
This method was similar to the Method No. 3 noted above. The difference was that the magnesium ions were introduced into the reaction vessel by way of the buffer instead of the titrant (Titrant No. 3 - Buffer No. 2). The data of Table V showed that this method was superior to the Methods No. 3 and No. 4 with low hardness and with samples having no magnesium present. With standardization of the EDTA titrant against a pure calcium solution, the same titrant strength factor could be used in the calcium hardness titration (see Table XIII).

Table V. EDTA Method—Magnesium Salt of EDTA in the Ammonia Type Buffer

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
200.0	404.0	604.0	604.0	0.0
200.0	400.0	600.0	601.2	+1.2
400.0	199.4	599.4	597.1	-2.3
203.6	111.5	315.1	316.2	+1.2
100.8	38.3	139.1	138.0	-1.1
50.8	0.0	50.8	50.9	+0.1
9.5	0.0	9.5	10.6	+1.1
			Av. dev.	1.0

Method No. 6. EDTA Method, Hach Odorless Buffer Containing Complexed Magnesium (26)

This method was similar to Method No. 5 in principle. The buffer was not ammonia in type, but did contain the magnesium salt of EDTA (Buffer No. 3). This method appeared to have all of the advantages of Method No. 5. In addition, the method had a more stable buffer which was not objectionable from an odor point of view. Results are shown in Table VI.

Method No. 7. EDTA Method, Monoethanolamine, Hydrochloric Acid Buffer Containing Complexed Magnesium (37)

The results are shown in Table VII. This method appeared to be very similar to the Method No. 6 in performance. The buffer preparation (Buffer No. 4) method used was not exactly the same as that described in the work of Patton and Reeder (37), but it was believed that the resultant reagent should be the same. There was some indication that the Method No. 6 buffer (Hach odorless buffer) was slightly superior as to buffer and inhibitor capacity.

Method No. 8. EDTA Method, MonoVer (26)

The commercially available reagent MonoVer which consisted of indicator, odorless buffer containing the magnesium salt of EDTA, and inhibitor was used with EDTA (Titrant No. 3) which contained no magnesium. The results are presented in Table VIII. It appeared that this reagent method was as satisfactory as Methods No. 5, 6, and 7. The method had the advantage from the standpoint of convenience in that only one addition of reagent needed to be made before titrating. In effect, it appeared that this procedure was the same as Method No. 6, except that the indicator was added separately in Method No. 6.

Table VI. EDTA Method—Odorless Buffer (Hach Chemical Co.)

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
100.0	412.4	512.4	514.6	+2.2
200.0	618.6	818.6	819.5	+0.9
845.1	57.9	903.0	904.2	+1.2
845.5	58.0	903.5	903.2	-0.3
			904.4	+0.9
845.3	57.7	903.0	901.7	-1.3
			901.3	-1.7
845.2	27.6	872.8	869.1	-3.7
845.2	31.2	876.4	875.4	-1.0
			876.6	+0.2
845.3	29.8	875.1	874.4	-0.7
			873.6	-1.5
845.3	58.4	903.7	906.5	+2.8
845.2	57.2	902.4	902.8	+0.4
			903.6	+1.2
203.6	111.5	315.1	317.7	+2.6
100.8	38.3	139.1	140.0	+0.9
50.8	0.0	50.8	52.0	+1.2
9.5	0.0	9.5	10.1	+0.6
			Av. dev.	1.3

Table VII. EDTA Method—Patton and Reeder Buffer

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
203.6	111.5	315.1	314.0	-1.1
100.8	38.3	139.1	138.0	-1.1
50.8	0.0	50.8	52.1	+1.3
9.5	0.0	9.5	8.5	-1.0
			Av. dev.	1.1

Table VIII. EDTA Method—MonoVer (Hach Chemical Co.)

Hardness Taken as ppm $CaCO_3$			Total Hardness	Dev. from
Calcium	Magnesium	Total	Found	known
203.6	111.5	315.1	312.8	-2.3
100.8	38.3	139.1	138.2	-0.9
50.8	0.0	50.8	51.6	+0.8
9.5	0.0	9.5	10.1	+0.6
			Av. dev.	1.1

C. Buffer Studies

1. Introductory Comments

The various buffers were studied to determine the pH value created, their stability, acid and base capacity, and their ability to take care of usual or important interferences.

2. Stability Studies

An accelerated method of study was used. Each buffer solution was placed in a 250 ml flask and held at 90°F in a temperature-controlled water bath. In each case, 150 ml of each buffer were used. Air was bubbled through the solutions by slight suction at the rate of about 800 ml per minute. At various intervals 1 ml of the buffer was withdrawn and added to 50 ml of demineralized water and the resultant pH measured using a glass electrode pH meter. The results of the study are given in Fig. 1.

It was apparent from Fig. 1 that the Hach odorless buffer solutions and the Patton-Reeder buffer were much superior to the ammonia-type buffer solutions as indicated by this test. The borate type buffer of Betz did not change much in pH effect, but it did deteriorate in normal use because of the sulfide present. The pH value was well above 10, and its normal shelf life was not good probably because of the oxidation of the sulfide.

The dry powder buffers were stored and used normally in the laboratory and no deterioration was noted over a nine-month period.

3. Buffer Capacity

a. Acid and Base Titration

The recommended amount of each buffer, both powder and liquid type, was added to 50 ml of demineralized water and the pH determined. Also the pH value was determined as acid was added and as base was added to the buffered water. Glass electrode pH meter was used in each case. The data are presented in Fig. 2 and Fig. 3.

It can be seen that the borate buffer gave a high pH value (10.7) to start with and that it has almost no buffer capacity either to acid or base.

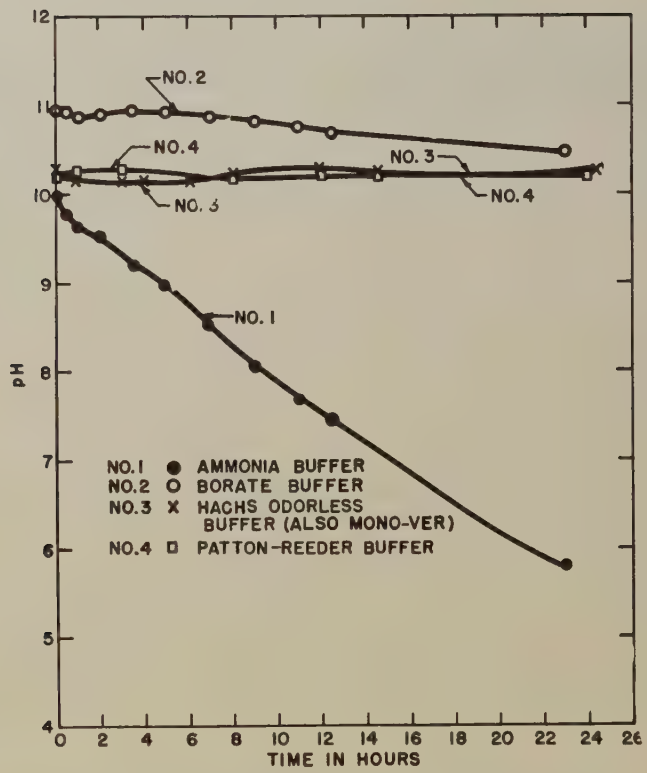

FIG. 1 STABILITY STUDY OF pH 10 BUFFERS (AT 90.8°F WITH AIR BUBBLING THROUGH 150 ml OF BUFFER AT 800 ml/min)

It was noted that the two powder buffers, UniVer I and UniVer II, appeared to have better buffer capacity both to acid and base than any of the liquid buffers when used as prescribed. The Hach odorless buffer solutions (Odorless Buffer and MonoVer solution give identical results) appeared to have a little more capacity than the odorless Patton and Reeder buffer.

b. Effect of Acid from Standard Calcium Chloride Solution

In order to obtain a very practical observation of the buffer capacity of the various buffers, a further study was made. The various buffers were added to 50 ml of water samples made up by adding varying amounts of a 1000 ppm calcium chloride (as $CaCO_3$) solution that was prepared from primary standard calcium carbonate by the regular method (dissolving in about 10 ml of 3 N hydrochloric acid, boiling, cooling, and neutralizing with dilute ammonium hydroxide using methyl red as the

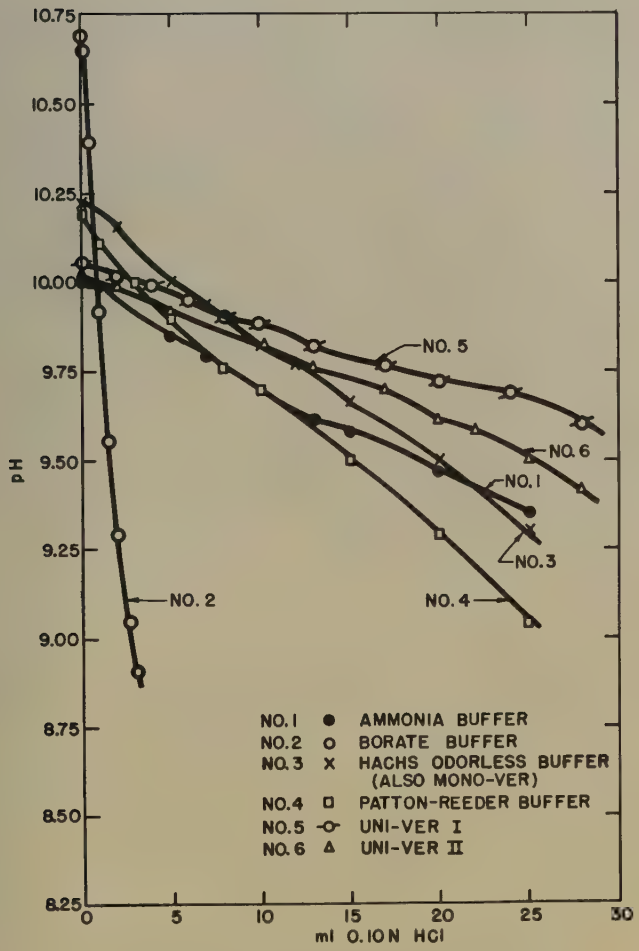

FIG. 2 POTENTIOMETRIC TITRATION OF BUFFERED SOLUTIONS WITH DILUTE ACID

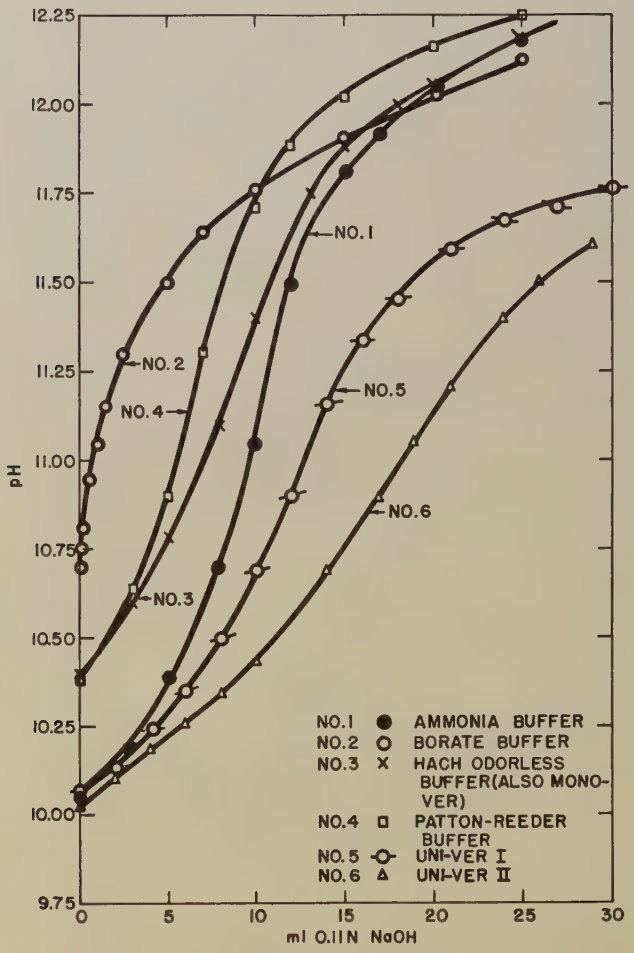

FIG. 3 POTENTIOMETRIC TITRATION OF BUFFERED SOLUTIONS WITH DILUTE BASE

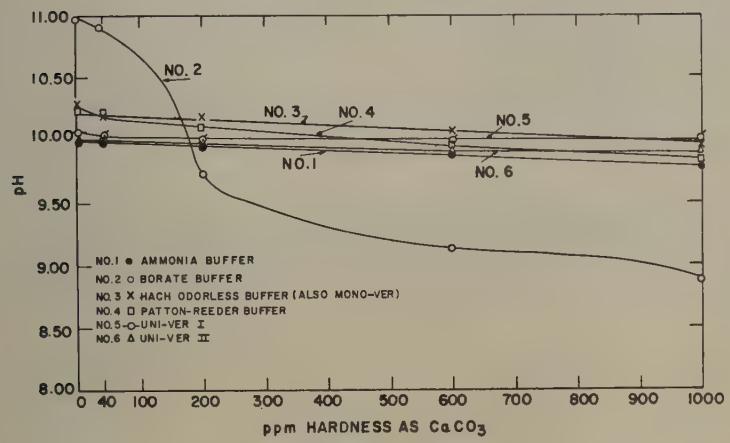

FIG. 4 RESULTING pH OBTAINED BY ADDING PRESCRIBED AMOUNT OF BUFFERS TO HARDWATERS (BEFORE TITRATION)

indicator). The data are presented in Fig. 5. The zero ppm water was demineralized water only. The 40 ppm water was made by pipetting 1 ml of the 1000 ppm calcium chloride solution into the beaker and diluting to 50 ml volume with demineralized water. The 200 ppm water sample was prepared by pipetting 5 ml of the above described calcium chloride solution into the beaker and diluting to 50 ml volume with demineralized water. In a similar manner the 600 ppm and 1000 ppm water samples were prepared.

It was apparent that the small amount of acid capacity of the calcium chloride solution affected all of the buffers. The borate buffer was again shown to be very ineffective. It appeared that the ammonia buffer held the pH the most nearly constant, however, both the Hach Odorless and the Patton and Reeder buffers did a comparatively good job, with the Hach Odorless type being slightly superior to the Patton and Reeder type.

c. Effect of Acid formed During Titration

Since during the titration each metal ion liberated two hydrogen ions, a further study was made to show the buffer capacity situation and the pH existing at the end point of such titrations. The water samples of Part b above were titrated using the various titrants. The pH at the end point was determined and the data are shown in Figs. 5 and 6. The "base line" was the pH value before the titration started, and the other points were pH values found at the end point of each titration.

From Fig. 5 it was apparent that the borate type buffer was very ineffective and should never be used with a titrant that was not basic and carrying considerable base capacity. Fig. 6 showed that with the borate buffer the pH value changed considerably with the low hardness water and was very high at the beginning and the end of the titration. In the higher hardness samples, the starting pH was low (8.9 to 9.7) but holds fairly well during the titration owing to the base capacity of the titrant used.

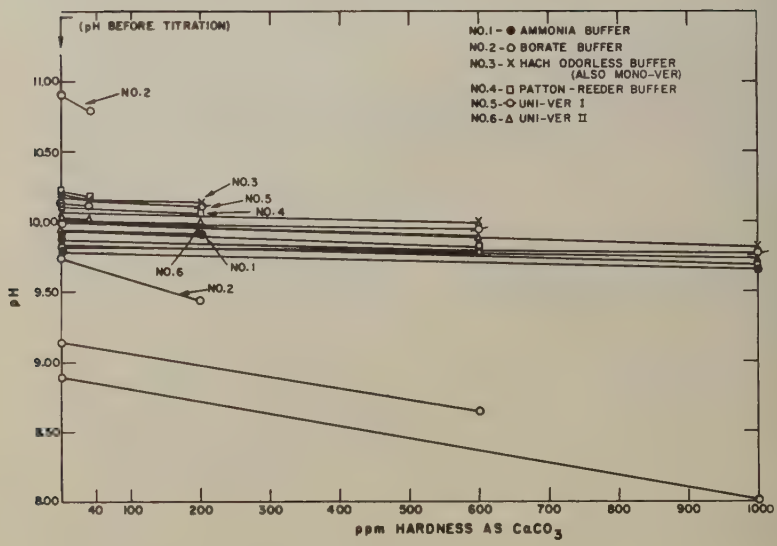

FIG. 5 pH DROP OF BUFFERED HARDWATERS IN TITRATION WITH EDTA # 3 (pH 5 TITRANT)

The other buffers all performed much alike with both type titrants. It was apparent that more than 1 ml of buffer might well be added in the standardization titration. To more fully consider the buffer's pH significance, refer to the pH effects on the accuracy of the indicator end point.

4. Inhibitor Capacity of Buffered Materials

Iron and copper were the two most common and serious interfering ions from the standpoint of destroying a good end point. To study the buffer's inhibitor capacity, the usual typical titration was used with diluted powder F-241 indicator added except where the indicator was a part of the buffer preparation. These latter cases were MonoVer, UniVer I, and UniVer II. The data obtained are given in Table IX. This table showed that all but the borate buffer and UniVer II handled up to about 25 ppm of iron. Copper gave a poor end point at very low values when the ammonia type buffers and the Patton and Reeder type were used. All of the others handled a reasonable amount of copper, but the UniVer I powder was capable of handling large amounts of copper.

D. End point pH Effect.

1. Introduction

It was apparent that the pH obtained at the end point of a titration can vary considerably (see Figs. 5 and 6). In order to evaluate the importance of this occurrence, the following studies were made.

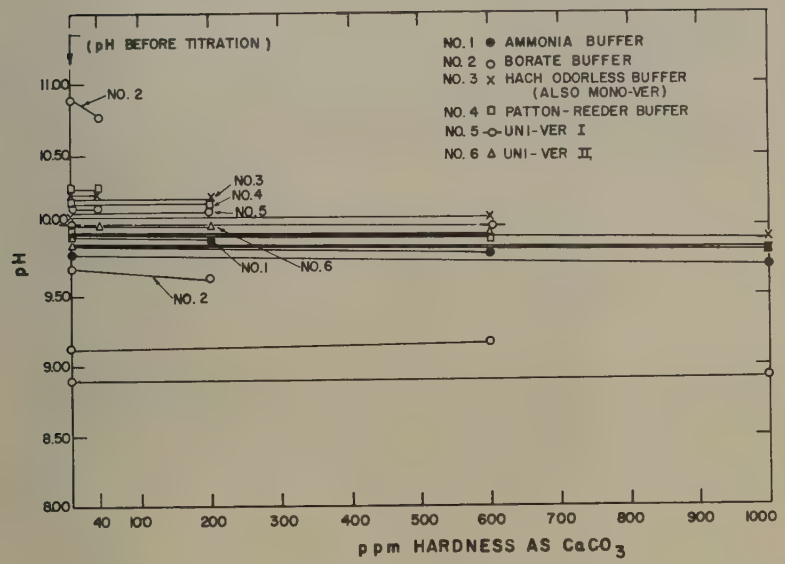

FIG. 6 pH DROP OF BUFFERED HARDWATERS IN TITRATION WITH EDTA #2 (NaOH IN TITRANT – BETZ TYPE)

Table IX. Inhibitor Capacity of Buffers for Iron and Copper. (water sample contains 100 ppm Ca and 20 ppm Mg expressed as ppm $CaCO_3$)

Buffer		Allowable Interference Concentration in ppm		
		Fe^{++}	Fe^{+++}	Cu^{++}
No. 1	Ammonium hydroxide Ammonium chloride	25	25	0.02
No. 2	Ammonium hydroxide Ammonium chloride + complexed magnesium	25	25	0.02
No. 3	Odorless nonvolatile buffer of Hach Chemical Co.	25	25	0.10
No. 4	Monoethanolamine hydrochloric acid buffer of Patton & Reeder	25	25	0.05
No. 5	Borate-sodium hydroxide sodium sulfide buffer of Betz & Noll	10	10	20
No. 6	MonoVer liquid of Hach Chemical Co.	25	25	0.10
No. 7	UniVer I powder of Hach Chemical Co.	25	25	2000
No. 8	UniVer II powder of Hach Chemical Co.	1	1	5

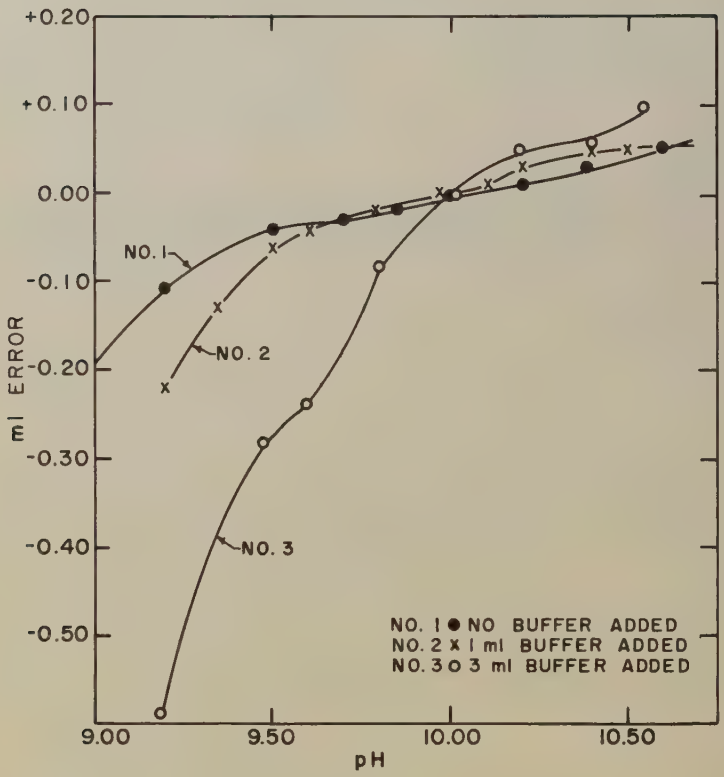

FIG. 7 EFFECT OF pH UPON END POINT OF F-241 USING THE AMMONIA BUFFER

2. pH Effect with Very Little Buffer Present

In this study, a 25-ml portion of the standard calcium chloride solution was introduced into the titration vessel. The magnesium salt of EDTA was added to make the amount of magnesium present the same as though 1 ml of ammonia buffer containing the magnesium salt of EDTA had been added. In this case no addition of ammonia buffer was made. Dilute ammonium hydroxide was added in sufficient quantity to the water sample to give a pH of about 9 to the end point. After the end point thus reached was recorded, additional dilute ammonium hydroxide was added to raise the pH slightly. When this was done the end point was shown not to be reached at this higher pH and more titrant (Titrant No. 3) was introduced until the clear blue color change was again reached. This process was repeated until Curve No. 1 was obtained in Fig. 7.

3. pH Effect with 1 ml of Buffer

One ml of the ammonium type buffer was added to the water sample and then sufficient hydrochloric acid added to give low pH at the end point. The titrant was the same as above (Titrant No. 3). After the end point was obtained, dilute ammonium hydroxide was added to raise the pH. The end point would then fade out and additional titrant added to again reach the blue color change. In this manner, Curve No. 2 of Fig. 7 was obtained.

4. pH Effect with 3 ml of Buffer

The same procedure as with the 1 ml of buffer study above was followed except stronger acid and base were used in adjusting the pH to the desired value.

5. Discussion of Fig. 7 Results

The correct end point was chosen as that obtained at pH 10. It was apparent that low results were obtained in a titration if the pH is below 10 and high results were obtained if the pH was above 10. It appeared that less error resulted on the high pH side of 10 than on the low side. Perhaps it should be pointed out that although the accuracy of the end point was more pH dependent when larger amounts of the buffer were present, with more buffer of an effective type added the pH was more properly controlled.

Data given in Table X showed that more than 1 ml of the various buffers can be added in the standardization titration giving better pH control at the end point. Here again the borate buffer was shown to be ineffective in controlling the pH. A large excess of the ammonia buffer did not raise the pH very much in these titrations, and the end points were still satisfactory. The odorless buffers did give high pH values when used in excess, and with the 5 ml of buffer present the end points were not as sharp as usual. From this study it appeared that when 1 ml of the buffer being used did not give satisfactory pH control in a standardization titration, 2 or 3 ml of buffer could be used, and satisfactory end points would be obtained giving more accurate results than if titrating with less pH control was allowed.

6. pH Effect with Different Type Buffers

A study using the different type buffers was made using 1 ml of buffer in each case for the liquid buffers and about 1 gram of the dry powder materials. The results are shown in Fig. 8. The ammonia type buffer (Curve No. 2) of Fig. 7 and Fig. 8 were the same and common comparison curve. It appeared that all of the buffers gave satisfactory results between pH of about 9.8 and 10.2 and that the Hach odorless buffers and the Patton and Reeder buffer were superior to the ammonia type and borate type buffers, especially on the higher than pH 10 side.

7. Sodium Cyanide pH Effect

The addition of relatively large amounts of sodium cyanide to a buffered solution gave an apparent increase in pH. A 25 ml sample of Iowa State College water was diluted to 50 ml buffered with varying amounts of the ammonia buffer, and titrated to the end point. The pH value was

Table X. Titrations of 25 ml of the Calcium Chloride Solution with Increasing Amounts of Buffer Added

Buffer	Final pH	ml EDTA	End points Remarks
1. Ammonia buffer containing complexed magnesium*			
1 ml	9.70	24.97	Sharp
3 ml	10.01	25.01	Sharp
5 ml	10.15	25.04	Sharp
2. Borate buffer*, **			
1 ml	8.04	24.10	Broad and indistinct
3 ml	9.54	24.94	Fairly sharp
5 ml	9.97	24.95	Sharp
3. Borate buffer***			
1 ml	8.90	24.02	Very broad and indistinct
3 ml	10.57	24.94	Somewhat better but still very faint
5 ml	11.12	25.12	Somewhat better but still very faint
4. Hach odorless buffer*			
1 ml	9.85	24.97	Sharp
3 ml	10.33	24.97	Sharp
5 ml	10.41	25.91	Less distinct
5. Patton & Reeder buffer*			
1 ml	9.75	24.97	Sharp
3 ml	10.28	25.01	Sharp
5 ml	10.35	25.01	Less distinct

* EDTA titrant No. 3 was used in the titrations.

** The complexed magnesium salt of EDTA was added to the beaker to sharpen the end point.

*** EDTA titrant No. 2 was used in the titrations, and no complexed magnesium salt of EDTA was added.

observed before varying amounts of sodium cyanide were added, after the sodium cyanide was added, and at the end point. The results are shown in Table XI. The sodium cyanide additions showed an apparent increase in the pH value at the end point, but this occurrence did not result in high results as would be predicted from the pH effects on the end point.

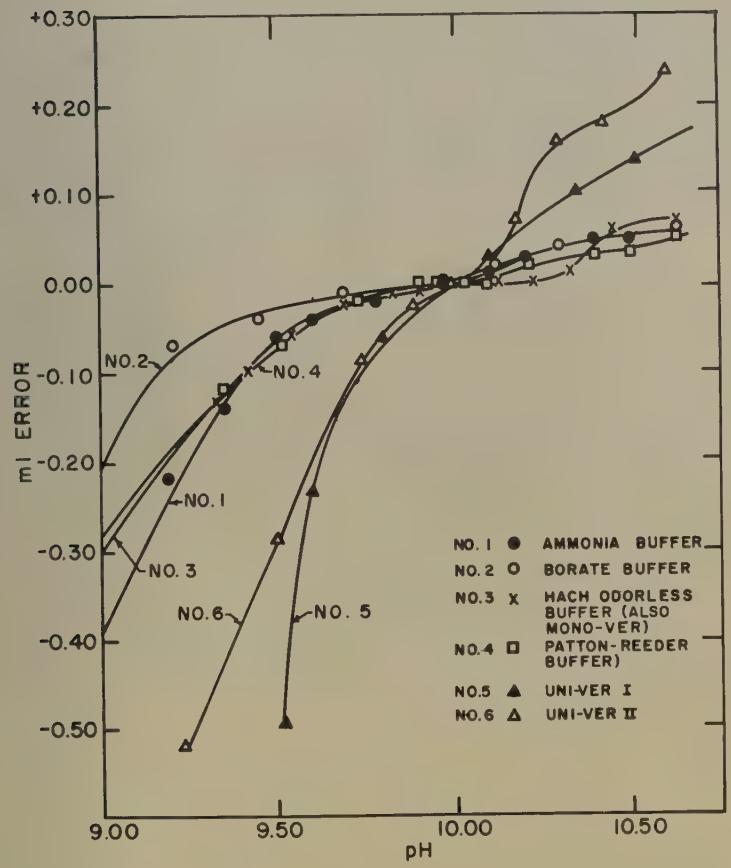

FIG. 8 TITRATION OF 25 ml OF $CaCl_2$ SOLUTION AT VARYING pH WITH NORMAL ADDITION OF BUFFERS

E. Interferences

Table XII gave the various interference limits as determined using the ammonia type buffer and F-241 indicator with no inhibitor substances present as well as with various inhibitor formulations added. The interference was manifested by a poor or unsatisfactory end point in the typical titration or when the interfering material titrated as hardness. It was apparent that the APHA "inhibitor a" solution handled a reasonable amount of copper and iron as does "inhibitor b," but that the large amount of sodium cyanide (0.25 gram added as dry powder) did a superior job. The presence of very low concentrations of zinc and cadmium gave high results with "inhibitor a" added, but the large addition of sodium cyanide removed these interferences quite well.

Table XI. Effect of Sodium Cyanide Addition with Buffer Present on the Alkalinity of the Solution and Hardness Results of the Titration*

Amount of buffer**	Amount of NaCN added	pH before titration (no NaCN)	pH before titration (with NaCN)	pH after titration	ml of titrant
1 ml	---	10.10	---	10.02	10.46
	---	10.10	---	10.05	10.44
	0.25 g	10.10	10.20	10.09	10.35
	0.25	10.10	10.22	10.12	10.35
	0.25	10.10	10.20	10.10	10.38
	0.50	10.10	10.28	10.19	10.39
2 ml	0.25 g	10.12	10.14	10.09	10.42
	0.25	10.10	10.12	10.10	10.41
3 ml	0.25 g	10.10	10.12	10.05	10.38
	0.25	10.12	10.14	10.11	10.38
5 ml	--	10.20	---	10.09	10.43
	0.25 g	10.20	10.21	10.20	10.38
	0.25	10.22	10.22	10.18	10.39

* Sample consisted of 25 ml of Iowa State College water.
** The ammonia type buffer was used.

Cyanide water solutions of more than 1% were not stable. A study of various strength cyanide solutions was made, and the data are presented in Fig. 9. The decomposition was indicated by development of a yellow color as the solutions decomposed. It would appear that a 1% solution was not limited in its stability but that higher concentrations were not practical for prolonged storage.

F. Indicator Stability

Diskant (15) studied the stability of F-241 indicator in various solvents. In view of his work no special study was made. Our general impression was that the dry powder types used as such had the best use-shelf life. F-241 methyl alcohol was used in our study and was satisfactory over a five-month period. MonoVer reagent appeared to have a use-shelf life of about the same as F-241 in methyl alcohol.

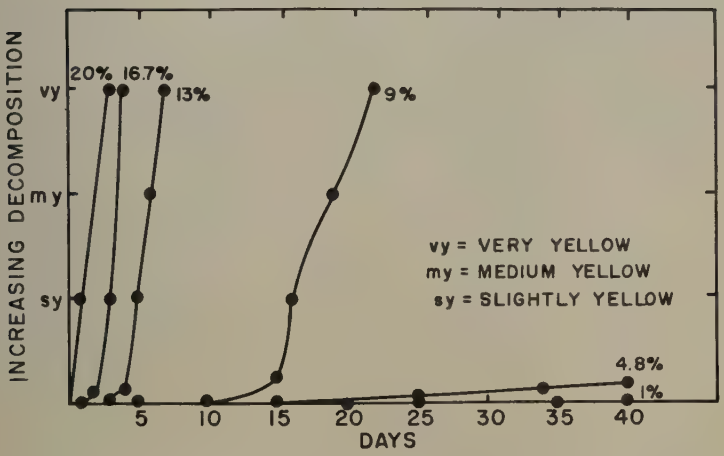

FIG. 9 STABILITY STUDY OF NaCN SOLUTIONS

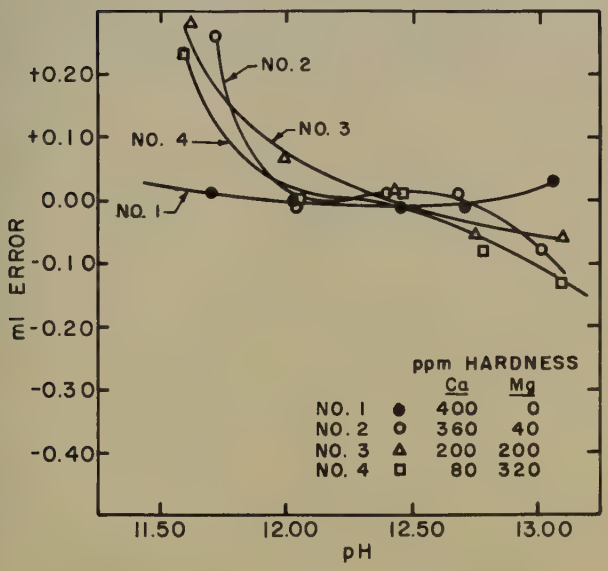

FIG. 10 TITRATIONS FOR CALCIUM HARDNESS WITH MUREXIDE

Table XII. Allowable Concentration of Interfering Ions Using F-241 Indicator and Ammonia Type Buffer*

Ion	No inhibitor	Inhibitor a (0.01 g NaCN)	0.25 g NaCN	Inhibitor b (Na_2S)	Borate buffer
Fe	25	30	60	10	10
Cu	0.02	50	3000	20	20
Cd	0**	0**	50	25	25
Ni	0.1	100	400	0.1	0.1
Co	0.1	80	400	0.1	0.1
Zn	0**	0**	200	200	40
Mn	0.3	1	1	1	1
Al	20	20	20	20	20
Pb	8	8	8	20	20

* The water sample contained 400 ppm of calcium and 200 ppm of magnesium as ppm of calcium carbonate.

** The ion titrated as hardness.

V. EXPERIMENTAL - CALCIUM HARDNESS

A. Introduction

In recent years several indicators to be used in calcium hardness titrations have been introduced. The early one was murexide (ammonium purpurate) and was proposed as a tentative standard method in the 10th Ed. of "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes" (43). The titrant recommended was either of the EDTA titrants mentioned in connection with the total hardness procedures.

Since magnesium chloride was added to one titrant but not to the other titrant (43) some confusion developed in the use of these titrants with regard to the calculation of the results. With the titrant containing magnesium a different strength factor was obtained if the titrant was standardized using F-241 indicator in the total hardness procedure and then used for calcium hardness in which the magnesium was not complexed at the end point. The magnesium ion for the total hardness titration can be readily introduced with the buffer, and this modification made it possible to use one titrant (as Titrant No. 3) with the same strength factor for both the total hardness and calcium hardness.

The calcium hardness indicators recently introduced and available were studied to evaluate them. The Patton and Reeder indicator was not available and was not studied. Interference studies also appeared to be something that should be investigated.

B. Standardization Using Various Indicators

Twenty-five ml of the standard calcium chloride solution were used in each of the titrations. Twenty-five ml of demineralized water and one ml of 8 N sodium hydroxide were added in the calcium hardness titrations to make the solution basic. The ammonia buffer containing magnesium was used with the F-241 indicator, and results used as the correct strength evaluation of the titrant. The apparent pH at the end of the calcium indicator titrations was measured using Beckman type E glass electrode. The results obtained are presented in Table XIII.

Table XIII. Comparison of Standardization Titrations with various Indicators

Indicator	pH adjusted with	ml EDTA	Dev. from Av.
F-241	3 ml ammonia buffer	25.01	+0.01
		25.02	+0.02
		24.97	-0.03
	(pH 10.01)	24.99	-0.01
		25.00	0.00
		av. 25.00	av. 0.01
Murexide	1 ml 8N NaOH	24.99	-0.02
		25.01	0.00
	(pH 12.65)	25.00	-0.01
		25.01	0.00
		25.03	+0.02
		av. 25.01	av. 0.01
CalVer II	1 ml 8 N NaOH	24.95	-0.02
		24.97	0.00
	(pH 12.65)	24.99	+0.02
		25.00	+0.03
		24.96	-0.01
		av. 24.97	av. 0.02
Calcon	1 ml 8 N NaOH	24.99	+0.01
		24.97	-0.01
	(pH 12.65)	24.95	-0.03
		24.97	-0.01
		25.03	+0.05
		av. 24.98	av. 0.02
Calcein	1 ml 8 N NaOH	24.99	-0.02
		25.01	0.00
	(pH 12.65)	25.03	+0.02
		25.02	+0.01
		25.01	0.00
		av. 25.01	av. 0.01

It can be seen that nearly the same results, within experimental error, were obtained in each of these standardization titrations.

C. Indicator Evaluation with Varying pH and Magnesium to Calcium Ratios

1. Introduction

In order to obtain a deeper insight into the behavior of the various calcium indicators, a series of experiments were conducted with varying ratios of magnesium and calcium present and with varying amounts of sodium hydroxide added to the solution to be titrated. A standard solution of calcium chloride and of magnesium chloride was used as a source of these two ions. They were prepared in the usual manner with the excess acid neutralized to methyl red indicator. Appropriate aliquots of the calcium and magnesium solutions were taken and diluted to 50 ml with demineralized water before the varying amounts of sodium hydroxide were added. Titrant No. 3 was used in each case. The pH at the end point of the titration was determined using the Beckman type E glass electrode.

The data are presented in Figs. 10, 11, 12, and 13. The titrant was standardized with F-241 at pH 10 using the ammonia buffer. Therefore, the "error in ml" indicated in Figs. 10 to 13 was the deviation in ml from what the amount of titrant should have been for the calcium present. The calcium present varied from 80 ppm to 400 ppm, thus the amount of titrant actually used was in the 2 ml to 10 ml range.

2. Murexide Indicator

Results with this indicator are shown in Fig. 10. Curve No. 1 showed the results of the titration when only calcium was present. With no magnesium ions present in the water sample, fairly sharp end points appeared giving quite accurate results except in the case where the pH was over 13.00. Here the color change was broad and indefinite and results were slightly high. With increasing concentration of magnesium and decreasing concentration of calcium present in the water samples No. 2 to No. 4, the best results were obtained between pH of 12.00 and 12.70. With higher pH values the end points were very broad and faint, and results were low. Below a pH of 12, the end points were not permanent, and high titration values were obtained. In general the sharpest end points were noted at pH values of about 12.50 or somewhat lower.

3. CalVer II Indicator

The data for this indicator are shown in Fig. 11. With no magnesium present accurate results were obtained over a wide pH range. In these titrations the color change was not as pronounced as when magnesium was present in the water sample, but it could be noted without any difficulty. With increasing amounts of magnesium and decreasing amounts of calcium in the samples No. 2 to 4, the curves indicated that pH values above 12.30 would give quite reasonable results using this indicator. Sharp end points were noted in the pH range of 12.40 to 13.10. The final color change of the indicator appeared to be sharpest at pH values near 12.40 or somewhat higher. Generally the end points became faint and

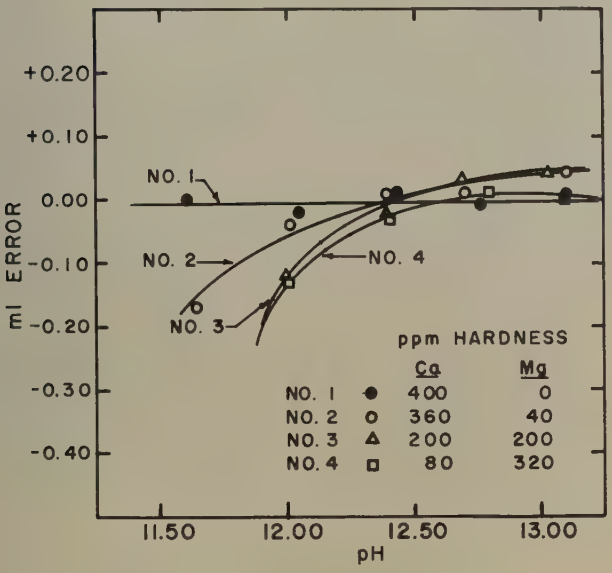

FIG. 11 TITRATIONS FOR CALCIUM HARDNESS WITH CAL-VER II

broad with pH values near 12.00 especially with the greater concentrations of magnesium present. With the pH below 12.00, the end points were usually quite faint and premature or failed completely.

4. Calcon Indicator

Data for this indicator are shown in Fig. 12. In the absence of magnesium accurate results were obtained only with pH values above 12.40 as indicated by the curve for sample No. 1. The end points were generally fainter in the absence of magnesium. High titration values were found with magnesium present. It appeared that the magnesium hydroxide precipitate had adsorbed some of the indicator-calcium complex causing the solution to appear slightly violet even past the equivalence point. The best results with magnesium present were noted with pH values between 12.40 and 12.70. With a pH of 13.10 the end points were sluggish, and the tendency was to titrate further past the equivalence point. With pH of 12.00 the end point reached was not permanent and more EDTA titrant was needed to reach the blue color once more.

Authors (3, 28, 32) have reported more successful titrations of calcium in the presence of magnesium with this indicator than were noted here. However, they employed either a special buffer system as diethylamine in place of the sodium hydroxide addition or added gelatin with the indicator to improve the end point.

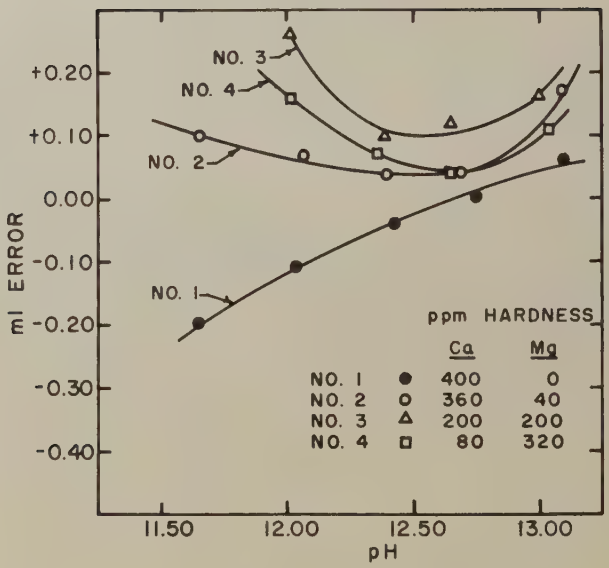

FIG. 12 TITRATIONS FOR CALCIUM HARDNESS WITH CALCON

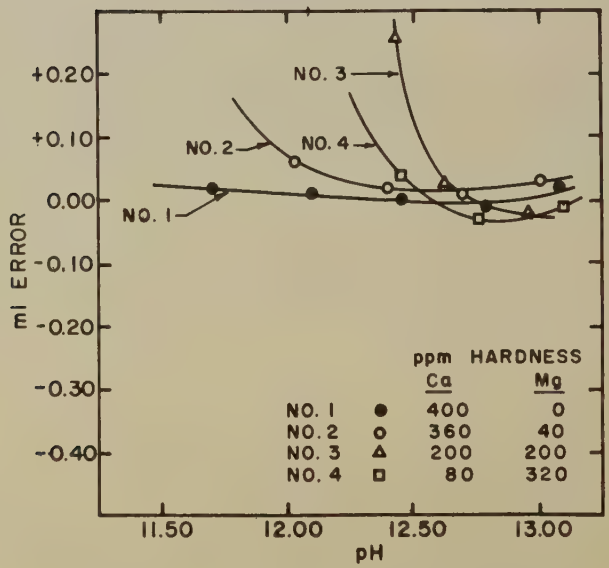

FIG. 13 TITRATIONS FOR CALCIUM HARDNESS WITH CALCEIN

5. Calcein Indicator

Data for this indicator are shown in Fig. 13. This indicator solution had a slight calcium blank, and in all titration values given, this blank was taken into account. In the absence of magnesium results were relatively consistent throughout the pH range studied. However, with the highest pH values the end points were broad, and with pH below 12 the color change noted was very faint. In the presence of magnesium, the end points were generally not permanent, and additional EDTA titrant was usually required after the first color change had appeared. This occurrence was more serious with greater concentrations of magnesium present and with smaller amounts of base added. From Fig. 13 it appeared that in general the optimum pH range for titrations with magnesium present would be in the pH region of 12.60 to 13.00 for best results using this indicator.

6. Summary

Of these indicators, murexide and CalVer II appeared to perform the most satisfactorily over wider pH ranges with varying amounts of magnesium present. Calcein gave comparable results if the pH was above 12.60. Calcon indicator was observed to have the narrowest optimum pH range. CalVer II and Calcon indicators gave the most pronounced color changes of these indicators studied.

D. Calcium Hardness Interferences

A series of interference studies with the calcium indicators was performed. Increasing amounts of various interfering ions were added to the water sample and titrated with and without inhibitor addition. Table XIV gave the data showing allowable concentrations of these ions in the various cases where satisfactory end points and results were still obtained. The data in Table XIV showed that the large addition of sodium cyanide (0.25 gram) was again more effective than "inhibitor a" in removing many interferences. It was noted in general that murexide and especially calcein were less influenced by interfering ions when no inhibitors were added. Calcon end points were affected more seriously by many interfering ions, especially by iron.

VI. GENERAL COMMENTS AND CONCLUSIONS

A. The EDTA titration methods offered more rapid analysis of hardness in water as compared to the gravimetric procedures with good accuracy still obtained. These titration methods were much superior to the soap titration method with respect to the hardness range which could be analyzed, the speed of titrations, and accuracy obtained.

B. The magnesium ion addition into the system appeared to be best accomplished by incorporating complexometrically neutral magnesium salt of EDTA with the buffer used. In this way a proper level of magnesium concentration was maintained even with samples of low hardness. With magnesium included in the EDTA titrant, poor end points may result if the water sample contained low hardness and no magnesium was present.

Table XIV. Allowable Concentration in ppm of Interfering Ions with Calcium Indicators *

Indicator	Inhibitor	Cu	Mn	Pb	Zn	Ni	Co	Al	Cd	Fe
Murexide	No inhibitor	5	10	10	5	25	25	40	5	16
	Inhibitor a	120	10	10	5	160	30	40	5	16
	0.25 g NaCN	4000	10	10	10	280	50	40	1000	16
	Inhibitor b	30	10	45	5	50	25	40	80	16
CalVer II	No inhibitor	1	3	10	10	1	1	30	5	12**
	Inhibitor a	40	3	10	10	160	1	30	5	16**
	0.25 g NaCN	4000	3	10	10	200	100**	30	800	16**
	Inhibitor b	40**	3	40**	10	5	1	30	100**	8**
Calcon	No inhibitor	1	3	10	10	1	1	12	2	1
	Inhibitor a	50	3	10	10	80	1	12	5	1
	0.25 g NaCN	4000	3	10	10	280	80	12	1000	1
	Inhibitor b	60**	3	35**	10	1	1	12	100**	2**
Calcein	No inhibitor	5	20	10	5	80	160	260	5	20
	Inhibitor a	35	20	10	5	120	300	260	5	30
	0.25 g NaCN	4000	20	10	10	160	400	260	1000	36
	Inhibitor b	35	20	100	5	100	25	260	100	20

* The water sample contained 100 ppm calcium and 20 ppm magnesium as ppm calcium carbonate.
** The titration was not to the normal blue end point.

C. If magnesium ions were added with the buffer, then only one standard EDTA titrant (which contained no magnesium) was needed in both total hardness and calcium hardness determinations. Standardization titrations of this titrant can be performed at both a pH of 10 with F-241 indicator and at a pH over 12.40 using various calcium indicators, and these titrations should agree within experimental error. Poorer end points were obtained in the calcium titrations when magnesium was present in the titrant especially when using CalVer II and Calcon indicators.

D. The borate type buffer procedure of Betz and Noll was found to be inadequate in several respects. No allowance for addition of magnesium in the standardization titration against standard calcium chloride solution was made, and the end point in these titrations was quite poor or failed completely. The borate buffer proposed had very little actual buffering capacity, and a special titrant of EDTA plus an appropriate amount of sodium hydroxide was necessary for any pH control to occur. In general the pH value obtained with 1 ml addition of this buffer varied from sample to sample, especially if initially the sample was slightly acidic.

E. The two odorless buffers studied were found to be much superior to the ammonia type buffer with regard to stability. Also these buffers had more inhibitory action toward iron and copper than was noted when using the ammonia buffer.

F. For the most accurate results when using F-241 indicator and the various pH 10 buffers, pH of 9.8 to 10.2 at the end point appeared to be the most satisfactory range. With large amounts of buffer present greater errors were noted with the same deviations from pH 10. These errors were larger with the pH below 10.

G. With slight acidity in the standard calcium chloride solution, 1 ml of buffer of various types did not usually meet the pH control requirements noted above. It appeared that 2 ml of buffer should be added to compensate for this acid capacity. In titrations of the natural, potable water samples usually 1 ml of the ammonia and odorless buffers was sufficient.

H. Sodium cyanide solutions of concentrations over 1% were noted to be unstable. Better removal of interfering elements such as iron, copper, cadmium, nickel, cobalt, and zinc was noted if approximately 0.25 gram of sodium cyanide was added to each solution before titration than when using the dilute cyanide solution, "inhibitor a," which was recommended by the APHA "Standard Methods" book. Addition of large amounts of cyanide was more conveniently accomplished by addition of sodium cyanide crystals rather than with concentrated solutions of cyanide which were unstable. This greater amount of sodium cyanide raised the pH at the end point slightly due to hydrolysis but accurate results were still obtained.

I. Dry indicator mixtures of F-241, which were found to be stable indefinitely, were preferred to any of the proposed solutions of the dye. Of the various solvents used in preparation of dye solutions, methyl alcohol was the most satisfactory one used in this work.

J. Study of the performance of the various calcium indicators indicated that close attention to the pH at the end point was important especially if magnesium was present in the sample solutions. In the presence of an appreciable amount of magnesium in the water samples, the results were less accurate. The accuracy in these cases also depended greatly on the pH at which the titration was being performed and which indicator was being used.

K. The data presented indicated that the general suitable pH ranges for the calcium indicators studied were as follows:

Murexide	12.20 to 12.70	Calcon	12.40 to 12.70
CalVer II	12.40 to 13.00	Calcein	12.60 to 13.00

For the most part, the best color changes of the indicators with all types of samples were observed with pH of 12.40 to 12.50 except for calcein which required a higher pH of 12.60 or greater. One ml addition of 4 N sodium hydroxide usually gave a pH close to 12.50.

Of these various indicators this observer rated CalVer II as giving in general the sharpest and most accurate end points with all types of water samples analyzed. Murexide and Calcein indicators were noted to be less influenced by interfering ions. For over-all performance the indicators were rated in this order:

1st	CalVer II	3rd	Murexide
2nd	Calcein	4th	Calcon

Presented at the 134th National Meeting of the American Chemical Society, Chicago, Illinois, September 7-12, 1958.

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MECHANISMS OF CARBON DIOXIDE FIXATION BY CELL-FREE EXTRACTS OF HETEROTROPHIC AND PHOTOSYNTHETIC BACTERIA*

C.L. Baugh, T. Myoda, D.S. Bates, and C.H. Werkman

Department of Bacteriology
Iowa State University, Ames

The Wood-Werkman reaction was proposed in 1935 to represent the empirical course of events in the assimilation of carbon dioxide by heterotrophic organisms (1). In this reaction CO_2 is fixed into pyruvate to form oxalacetate which is converted stepwise to malate, fumarate, and succinate.

The reaction was intended to represent a series of reactions and was not to present the finer details of the actual fixation. However, it was not anticipated that the course of the reaction would be altered, only that details would be added upon further study.

Kalnitsky and Werkman (2) while considering the energy requirements of the reaction postulated that phosphorylation of intermediates would be shown to occur. Subsequent studies have shown that phosphoenolpyruvate is the CO_2 acceptor in two distinct reactions for the fixation of CO_2 to form oxalacetate.

Bandurski and Greiner (3) demonstrated an irreversible addition of CO_2 to phosphoenolpyruvate by an enzyme preparation from spinach leaves according to reaction 1.

$$PEP + CO_2 + OH^- \rightarrow OAA + P_i \qquad (1)$$

Utter and Kurahashi (4, 5) have described a reversible CO_2 fixing reaction in enzyme preparations from bird liver. This reaction which proceeds according to equation 2 requires a nucleotide as a phosphate acceptor.

$$PEP + CO_2 + IDP \rightleftharpoons OAA + ITP \qquad (2)$$

These reactions are catalyzed by the enzymes phosphoenolpyruvate carboxylase and oxalacetic carboxylase respectively.

The presence of both enzymes has been reported in wheat germ extracts by Tchen and Vennesland (6) and more recently in extracts of Thiobacillus thiooxidans, a chemoautotrophic bacterium, by Suzuki and Werkman (7).

This paper presents some preliminary studies of CO_2 fixation utilizing cell-free extracts of two heterotrophic bacteria and a photosynthetic

The abbreviations used are phosphoenolpyruvate, PEP; oxalacetic acid, OAA; inosine triphosphate, ITP; inosine diphosphate, IDP; adenosine triphosphate, ATP; adenosine diphosphate, ADP; ethylenediaminetetraacetate, EDTA; and tris (hydroxymethyl) aminomethane, tris.

* Supported, in part, by funds of the U.S. Public Health Department.

autotroph, Nocardia corallina, Mycobacterium phlei, and Rhodospirillum rubrum.

MATERIALS AND METHODS

All reagents were commercial preparations; oxalacetic acid and phosphoenolpyruvate (tricyclohexylamine salt), California Foundation for Biochemical Research; sodium inosine triphosphate (ITP), sodium inosine diphosphate (IDP), sodium adenosine triphosphate (ATP), sodium adenosine diphosphate (ADP), Pabst Laboratories; $BaC^{14}O_3$, Oak Ridge National Laboratory.

Cell-free extracts were prepared by sonic oscillation in a 9-kc Raytheon oscillator at 4°C. Cellular debris was removed by centrifugation at 4°C in an International Refrigerated Centrifuge.

The growth conditions of the various organisms and the treatment of the cells prior to sonic treatment were as follows:

Nocardia corallina ATCC 4273 was grown in a medium containing 0.3% each of peptone and yeast extract for 24 hours at 30°C. The cells were harvested by centrifugation and washed 3 times with distilled water. Three grams (wet weight) of the washed cells were suspended in 15 ml of 0.1 M tris buffer of pH 7.4 and treated in the sonic oscillator for 30 minutes. The cell-free extract was dialyzed in a collodion or cellophane bag for 24 hours at 4° against 0.001 M tris buffer.

Mycobacterium phlei ATCC 10142 was grown in a modified Dubos medium which contained 1% glucose and no $CuSO_4$ for 48 hours at 37°C. The culture was vigorously aerated by a stream of air sterilized by passage through a sterile cotton filter. The cells were harvested by centrifugation, washed twice with distilled water, and 5 grams, wet weight, of the cells were suspended in 35 ml of 0.2M tris buffer. After sonic treatment for 25 minutes then centrifugation, sufficient $MnCl_2$ (1M) was added to the extract to make a 13% solution and the mixture stirred for 30 minutes at 4°C. The mixture was warmed in a 55°C water bath for 1 minute and centrifuged to remove the precipitated protein. The supernate was dialyzed against 0.05M tris buffer for 15 hours at 4°C.

Rhodospirillum rubrum ATCC 11170 was grown in 500 ml screw-top prescription bottles completely filled with a lactate medium described by Woody and Lindstrom (8) for 5 days at 30°C. The organisms were illuminated during this period with two 75 watt incandescent light bulbs at a distance of 50 cm. The cells were harvested by centrifugation, washed three times with distilled water, and suspended in 10 volumes of 0.1M tris buffer; 10 mg cysteine were added per gram of cells. This cell suspension was treated in the Raytheon oscillator for 5 minutes to obtain the extract. The extract was dialyzed in cellophane against 0.002M tris buffer (pH 7.4) for 70 hours.

The cell-free extracts of the three organisms were incubated in Warburg flasks with phosphoenolpyruvate (or oxalacetate) and $C^{14}O_2$ under various conditions. The reaction was stopped at the end of one hour by the addition of 0.2 ml of 50% trichloroacetic acid. The precipitated protein was removed by centrifugation and an aliquot pipetted onto a glass planchet. The sample was dried under a stream of air and the radioactivity fixed was determined immediately under a Geiger-Muller tube.

Oxalacetate containing C^{14} was shown to be formed in the following manner: carrier oxalacetate was added after the completion of the reaction and an equal volume of a saturated solution of 2, 4-dinitrophenylhydrazine in 2N HCl added. The mixture was allowed to set at room temperature for 30 minutes and then placed in the refrigerator overnight. The 2, 4-dinitrophenylhydrazone of oxalacetate was identified and shown to contain C^{14} by paper chromatography and radioautography.

RESULTS AND DISCUSSION

The results of the $C^{14}O_2$ fixation experiments are shown in Tables 1, 2, and 3.

Results obtained with each of the extracts show that $C^{14}O_2$ is fixed in the absence of added nucleotide when PEP is the substrate. Manganous ion fulfills the requirement for a divalent cation and cannot be replaced by magnesium. Pyruvate or pyruvate and ATP cannot replace PEP.

This fixation in the absence of added nucleotide is apparently irreversible in N. corallina and M. phlei since the exchange reaction (substrate:OAA) did not occur under these conditions in these extracts. This reaction is similar to the irreversible fixation of CO_2 by phosphoenolpyruvate carboxylase. However, no attempts were made to correlate the release of inorganic phosphate with the fixation of CO_2 due to the small amount of CO_2 fixed and the impurity of the enzyme preparation.

The fixation in each of the extracts was stimulated by the addition of a nucleotide when PEP is used as the substrate. The addition of a nucleotide (ITP) also allowed the exchange reaction to occur. This was considered evidence for the occurrence of a reversible, nucleotide-requiring CO_2 fixation similar to the one catalyzed by oxalacetic carboxylase.

This reversible reaction also required a divalent cation and in all three extracts manganese satisfied the requirement. However, in two of the extracts (N. corallina and M. phlei) manganese could be replaced by magnesium.

Since magnesium could replace manganese in the reversible fixation reaction and not in the irreversible fixation, this was considered additional evidence for the occurrence of two (at least) distinct fixation reactions in these two extracts and indicates that the fixation which occurred in the absence of added nucleotide was not due to residual nucleotide present in the extracts.

The fixation of $C^{14}O_2$ by extracts of Rhodospirillum rubrum in the absence of added nucleotide is variable and does not occur consistently under the experimental conditions. Since attempts to separate two fixation reactions so far have not been successful, it is not possible at this time to determine whether the fixation is due to residual nucleotide.

However, the presence of the reversible nucleotide-requiring fixation reaction is demonstrated by the stimulation of fixation upon the addition of nucleotides.

Since the Wood-Werkman reaction was known to be reversible it is probable that oxalacetic carboxylase represents a refinement of the original series of reactions in animal tissue. As yet the presence of

PEP carboxylase has not been reported in animal tissue, only in plants and in Thiobacillus thiooxidans, the chemoautotrophic bacterium.

The demonstration of two reactions for the formation of oxalacetate in the heterotrophic bacteria employed in this study suggests that in some bacteria the Wood-Werkman reaction could actually consist of a combination of these two enzymes. This conclusion is supported by the presence of both enzymes in Thiobacillus thiooxidans. It is interesting that the same pattern for CO_2 fixation into oxalacetate exists in the organisms representing the chemosynthetic, photosynthetic, and heterotrophic forms of life. This is the first report of the presence of these reactions in heterotrophic and photosynthetic bacteria.

Table 1. $C^{14}O_2$ fixation by cell-free extracts of Nocardia corallina

The complete system contained PEP, 3 μmoles; tris (pH 7.4), 100 μmoles; $NaHC^{14}O_3$, 5 μmoles (2×10^7 cpm); cell-free extract, 0.5 ml; $MnCl_2$ 5 μmoles; EDTA, 2 μmoles. Total volume: 2 ml. Gas phase: 100% N_2. Temperature 30°C.
Additions: IDP, ITP, OAA, 3 μmoles each; $MgCl_2$ 5 μmoles.

Omissions	Additions	Activity fixed (cpm/100λ)
1. none	none	835
2. PEP	none	3
3. $MnCl_2$	none	0
4. $MnCl_2$	$MgCl_2$	0
5. none	IDP	1421
6. $MnCl_2$	IDP	0
7. $MnCl_2$	IDP, $MgCl_2$	496
8. PEP	Pyruvate	20
9. PEP	Pyruvate, ATP	35
10. PEP	Pyruvate, ITP	53
11. PEP	OAA	2
12. PEP	OAA, ITP	336
13. PEP, Mn	OAA, ITP,	0
14. PEP	OAA, ITP, Mg	252
15. PEP	Malate, ITP	0

Table 2. $C^{14}O_2$ fixation by cell-free extracts of Mycobacterium phlei

The complete system was similar to that of Table 1 with the exception that no EDTA was included and 2.5 μmoles of $NaHC^{14}O_3$ (1 x 10^7 cpm) were used.

Omission	Additions	cpm/100λ
1. none	none	104
2. PEP	none	0
3. $MnCl_2$	none	0
4. $MnCl_2$	$MgCl_2$	0
5. none	IDP	470
6. $MnCl_2$	IDP, $MgCl_2$	74
7. PEP	OAA	0
8. PEP	OAA, ITP	3718
9. PEP, $MnCl_2$	OAA, $MgCl_2$	0
10. PEP, $MnCl_2$	OAA, $MgCl_2$, ITP	5280
11. PEP	Malate, ATP	0
12. PEP	Pyruvate	0

Table 3. $C^{14}O_2$ fixation by cell-free extracts of Rhodospirillum rubrum

The conditions were the same as in Table 1 except 0.2 ml of extract, 2.5 μmoles of $NaHC^{14}O_3$ (1 x 10^7 cpm), 5 μmoles cysteine, no EdTA, and 50 μmoles of potassium phosphate (pH 6.5) instead of tris were used.

Omission	Additions	cpm/100λ
1. none	none	64
2. PEP	IDP	11
3. none	IDP	300
4. $MnCl_2$	IDP, $MgCl_2$	13
5. PEP	OAA	41
6. PEP	OAA, ITP	802
7. PEP	Pyruvate, IDP	7
8. PEP	Malate	13

SUMMARY

Evidence is given for the presence of (at least) two different reactions for fixation of CO_2 into oxalacetate in cell-free extracts of N. corallina and M. phlei. One is an irreversible reaction similar to the one catalyzed by the enzyme phosphoenolpyruvate carboxylase and the other is a reversible nucleotide-requiring reaction similar to the reaction catalyzed by the enzyme oxalacetic carboxylase.

Extracts of R. rubrum were also shown to contain the reversible fixation reaction requiring nucleotides.

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